



THE AMERICAN JOURNAL  
OF PATHOLOGY





# THE AMERICAN JOURNAL OF PATHOLOGY

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## A COMPARISON OF THE INCLUSION BODIES OF FOWL- POX AND MOLLUSCUM CONTAGIOSUM \*

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With the demonstration of the infectious nature of the inclusion bodies of fowl-pox<sup>1</sup> it has seemed possible that inclusion bodies of other filtrable virus diseases might be used in similar experiments. Among these diseases is molluscum contagiosum, which closely resembles fowl-pox with regard to the large size and the distinctness of its intracytoplasmic inclusions. Furthermore, in a recent study of the subject, Goodpasture and King<sup>2</sup> have confirmed the observations of Lipschütz<sup>3</sup> and da Rocha Lima<sup>4</sup> that the inclusions of molluscum contain myriads of extremely minute round bodies — another respect in which molluscum and fowl-pox inclusions are similar. This observation, together with the fact that the molluscum bodies are of such large size, encouraged us in the hope that these bodies might be freed by tryptic digestion for purposes of inoculation, as were the fowl-pox inclusions. Accordingly, a series of experiments was undertaken, the results of which will be reported briefly. Since the gross and microscopic characteristics of molluscum contagiosum have been adequately described in textbooks and numerous papers on the subject, the molluscum body, which is the most characteristic feature of the lesion, may be considered without further description of the infected tissue.

\* Received for publication October 9, 1930.



## THE EFFECT OF TRYPSIN ON THE MOLLUSCUM BODIES

When a piece of tissue from a molluscum lesion is placed in 1 per cent trypsin, the shell of epithelium about each molluscum body digests away, freeing the body (Fig. 1). As may be seen, the bodies thus freed by digestion are oval or round in outline and exhibit a finely granular structure. In addition to this fine granulation, a sort of trabeculation of the bodies can be distinguished especially clearly in a preparation which has been teased out in saline (Fig. 2). These trabeculae were described by White and Robey <sup>5</sup> in 1902, the entire structure being aptly compared to a honeycomb. The authors described the formation of the trabeculae from the cytoplasm of the affected cell. That this explanation is true and that the trabeculae are actually a portion of the epithelial cell is indicated by their reaction to trypsin. A period of thirty minutes in the digesting solution causes the trabeculae to dissolve and allows the various compartments to disintegrate (Figs. 3 and 4). The molluscum bodies still retain their outline and consist of a jelly-like matrix in which the appearance of granulation is conspicuous. The matrix material, after digestion of the trabeculae, is extremely sticky and strings out like a piece of dough when it is touched with a glass or steel point. Active Brownian motion of granules may be seen in the gelatinous compartments, and the granules are easily expressed and many of them become free while still vibrating rapidly.

In their reaction to trypsin the molluscum bodies differ markedly from the firm, compact inclusion bodies obtained by the digestion of a fowl-pox lesion. The fowl-pox inclusions are protected from the action of trypsin, presumably by their lipoid content, and can be digested completely free from adventitious cellular material without interfering with their homogeneity. In order to get molluscum bodies similarly free from cellular material, the removal of their cytoplasmic trabeculae is necessary. With this removal the molluscum bodies are found to be composed of sticky masses and are rendered quite unfit for any sort of manipulation as units. Because of this stickiness, our attempted duplication of the inoculation experiments which were successfully performed in the case of fowl-pox had to be abandoned. Recently a technique has been evolved for inoculating minute portions of the fowl-pox inclusions.<sup>6</sup> This latter technique has not yet been attempted with molluscum, but we feel

that it would be practicable, and preferable to attempts to inoculate the entire inclusion bodies.

The gelatinous matrix of the molluscum bodies shows a much greater resistance to the digestive action of trypsin than is shown by the trabeculae. Furthermore, in any fresh preparation, whether the bodies are suspended in trypsin, normal saline or distilled water, the matrix exhibits the markedly granular appearance which has already been mentioned. This appearance is caused by the presence of innumerable Lipschütz granules — minute coccoid structures 0.25 of a micron in diameter within each inclusion. The granules were discovered by Lipschütz in smear preparations stained with Loeffler's flagella stain. He also observed them within the molluscum bodies in fresh preparations. At the present time the Lipschütz granules are recognized by many observers to be as characteristic of the molluscum lesion as are the molluscum bodies themselves, for, wherever the latter are found, there the Lipschütz granules can also be demonstrated. The granules are resistant to the action of trypsin to an even greater degree than the matrix material which surrounds them. In our experience, periods of five hours digestion have made no demonstrable change in their morphology and in all probability they would resist tryptic digestion for much longer periods.

#### THE EFFECT OF DISTILLED WATER

One of the striking characteristics of the inclusion bodies of fowl-pox is the marked swelling which they exhibit when placed in distilled water. It has been pointed out that this swelling continues until the original volume of the inclusions is increased two or three times.<sup>6</sup> Molluscum bodies, on the other hand, show little or no swelling under the influence of distilled water. The matrix of the molluscum body is probably completely permeable, since it promotes no swelling, while the lipoid material of the fowl-pox inclusion acts as a semipermeable membrane, permitting a marked imbibition of water.

Within the fowl-pox inclusions which have become swollen in distilled water may be seen myriads of minute bodies dancing about in rapid Brownian motion. These bodies, called Borrel bodies after their discoverer, are an exact counterpart of the Lipschütz granules of molluscum. In either disease the minute structures are coccoid in shape, 0.25 of a micron in diameter and react in similar fashion to

special stains <sup>7</sup> (Figs. 5 and 6). As may be seen they appear either singly, in diploid or chain form, or in masses. Lipschütz stated that the minute granules are readily differentiated from any impurity or precipitate which might settle on the slide, and, with regard to a carefully made preparation, we can completely corroborate this statement. Moreover, the great uniformity in the size and shape of the granules in the two diseases militates against the argument of those who contend that such granules are mere fragments of chromatin or keratohyaline material.

The most effective method of obtaining the minute elements well dispersed is, in the case of fowl-pox, to allow the digested inclusions to become swollen in a pool of distilled water on a slide and then allow the water to dry.<sup>6</sup> The relatively great tension of the thin film of water abruptly tears apart the lipoid material of the inclusion at the moment the water dries from its surface, leaving the Borrel bodies nicely dispersed (Fig. 6).

The inclusion bodies of molluscum not only show no imbibition when they are placed in distilled water, but, possibly because of their failure to swell in water, fail completely to be broken up when they are allowed to dry out of distilled water. The best preparations of the Lipschütz granules are obtained by making direct smears from the molluscum lesion, or by grinding the lesion, as will be described later.

### EFFECT OF TRITURATION

A study of the literature indicates that there is a difference in the readiness with which the fowl-pox and molluscum viruses may be passed through filters. Marx and Sticker,<sup>8</sup> and later Juliusberg<sup>9</sup> were successful in filtering fowl-pox virus through Berkefeld filters. Burnet<sup>10</sup> was able only occasionally to obtain passage through a Berkefeld filter and in no case through a Chamberland filter. Juliusberg,<sup>11</sup> on the other hand, obtained molluscum virus after passage through a Chamberland filter, and Wile and Kingery,<sup>12</sup> apparently without difficulty, passed active molluscum virus through a Berkefeld filter. It would seem that the molluscum virus filters somewhat more readily than does the fowl-pox virus.

Since the common mode of preparing virus material for filtration is that of thorough grinding in a mortar, we were interested to observe the different microscopic pictures presented by the smears of

ground material in the cases of molluscum and fowl-pox respectively. After grinding bits of molluscum tissue in saline in a mortar for periods of five minutes, smears of the resulting material show the Lipschütz granules to be well dispersed (Fig. 5). After a similar treatment of fowl-pox material the smears show irregular masses of débris with very few discrete, definite Borrel bodies. In the case of fowl-pox, grinding the diseased tissue evidently fails to liberate the Borrel bodies completely, while in molluscum the liberation and dispersion of the Lipschütz granules is practically complete. This difference in the reaction of the inclusion bodies of the two diseases to grinding may again be due to the lipoidal element in the inclusions of fowl-pox.

### COMMENT

Whether or not the readiness with which the Lipschütz granules are dispersed upon grinding the molluscum tissue is of importance in explaining the apparently greater filtrability of this virus cannot be stated definitely at this time. In the case of fowl-pox, evidence has been presented which would associate the Borrel bodies with the actual virus of the disease.<sup>6</sup> While no such evidence is at present available in the case of molluscum contagiosum, still the similarity of the Lipschütz granules to the Borrel bodies in size, shape and position within the inclusion points to the probability that the minute elements in the two diseases represent the viruses concerned. If such is the case, the ready dispersion of the Lipschütz granules upon grinding and the great difficulty met with in attempting to disperse the Borrel bodies afford an explanation of the relatively greater filtrability of molluscum.

Though the great similarity of Borrel bodies to Lipschütz granules has been pointed out, there is no intention in so doing of indicating a common etiology of the two diseases. The reverse has been demonstrated by numerous observers, and, in our own hands, the inoculation of fowl-pox on man and of molluscum on chickens has in no case given any suggestion of a positive result. Moreover, repeated intravenous injections of molluscum virus into chickens has failed, in our experience, to induce any immunity to subsequent infection with fowl-pox virus. That fowl-pox, particularly, is a highly specific disease is indicated by the fact that a single passage of the closely related pigeon-pox through fowls causes the virus, in most instances,

to lose its virulence for pigeons while still producing the typical fowl-pox lesion in chickens. If strains of the virus are specific for such closely related forms as fowls and pigeons, one would not expect the infectious agent of fowl-pox to cause disease in man. Several attempts to infect monkeys (*M. rhesus*) and other laboratory animals with molluscum virus have in our hands proved unsuccessful.

### SUMMARY

1. The inclusion bodies of molluscum contagiosum may be freed from surrounding cellular material by tryptic digestion.
2. Unlike fowl-pox inclusions, the molluscum bodies are found to be sticky and gelatinous after they have been digested. Because of this characteristic they cannot be manipulated readily with the Chambers microdissection apparatus.
3. The gelatinous matrix of the molluscum bodies has a markedly granular appearance due to the presence within it of myriads of Lipschütz granules — minute coccoid structures 0.25 micron in diameter. These granules are identical in size, shape and staining reactions with the Borrel bodies of fowl-pox. They are resistant to the action of trypsin.
4. The inclusion bodies of molluscum, on being placed in distilled water, show little or no swelling. Under similar conditions the fowl-pox inclusions swell markedly, due, probably, to their lipid material acting as a semipermeable membrane.
5. Trituration of the molluscum inclusions readily breaks them up into the component Lipschütz granules. Fowl-pox inclusions, similarly treated, fail to break up so readily into Borrel bodies. This difference in the reaction of molluscum and fowl-pox to trituration may afford an explanation for the relatively greater filtrability of the former.
6. Fowl-pox and molluscum contagiosum are apparently specific for fowls and man respectively, cross-inoculation experiments having proved unsuccessful. Attempts to transfer molluscum to monkeys and other laboratory animals were also unsuccessful.

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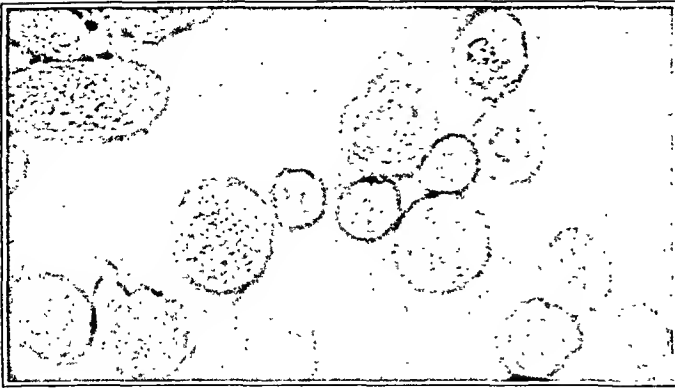
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## DESCRIPTION OF PLATE

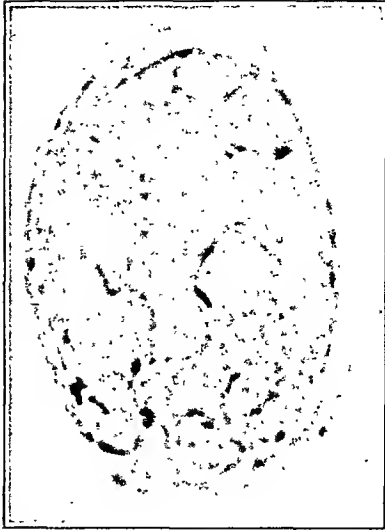
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### PLATE I

- FIG. 1. Inclusion bodies of *molluscum contagiosum* (molluscum bodies) freed from cells by tryptic digestion.  $\times 550$ .
- FIG. 2. Molluscum body in normal saline, teased free from surrounding cells. Note trabeculation within the inclusion.  $\times 1450$ .
- FIGS. 3 and 4. Molluscum bodies in trypsin with beginning digestion of trabeculae. Note finely granular appearance of matrix material.  $\times 1450$ .
- FIG. 5. Lipschütz granules of *molluscum contagiosum*. Morosow's stain. Photomicrograph taken with white light.  $\times 1860$ .
- FIG. 6. Borrel bodies of fowl-pox. Morosow's stain. Photomicrograph taken with white light.  $\times 1860$ .



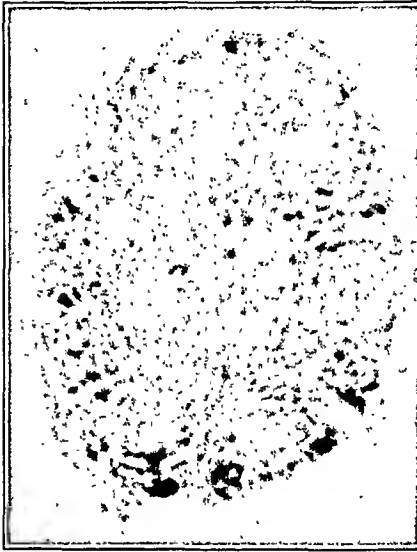
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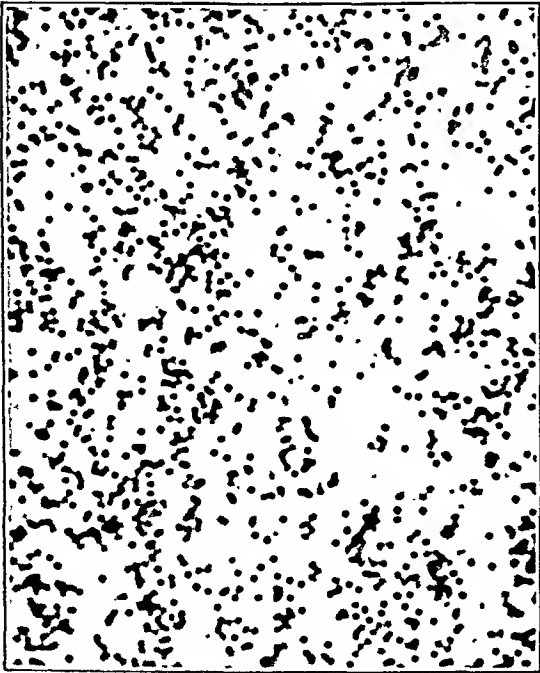
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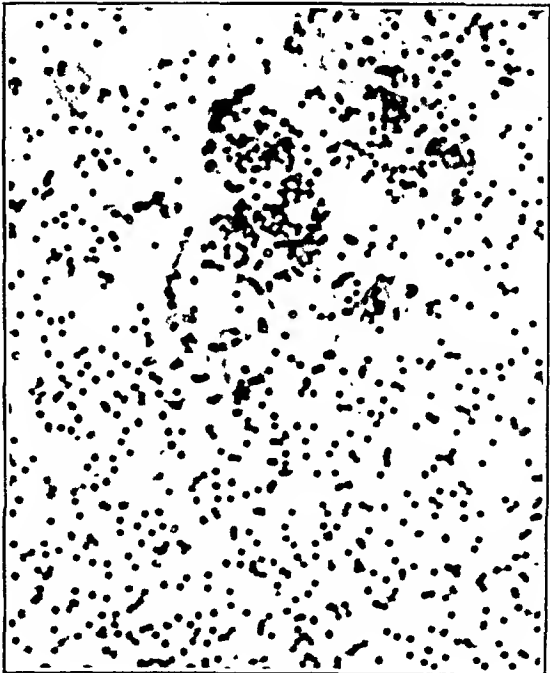


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5

Goodpasture and Woodruff



6

Fowl-Pox and Molluscum Contagiosum





## TRANSFORMATION OF SINUSOIDAL ENDOTHELIUM INTO THE ORDINARY CAPILLARY TYPE \*

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According to a prevalent view the capillaries of certain organs are lined by cells unrelated to ordinary endothelium. This conception is based largely on the common observation that the endothelium of the spleen, liver and bone marrow is actively phagocytic under natural and experimental conditions, in contradistinction to endothelium elsewhere which usually remains inactive. A related important characteristic of the endothelium of these organs is its affinity for colloidal dyes experimentally administered. In subcutaneous granulation tissue the author <sup>1,2</sup> found that the endothelium may be artificially stimulated to such a degree that it behaves in both of these particulars like the sinusoidal type. That all endothelial cells are potentially sinusoidal was concluded. The present experiments were aimed at a lessening of the functional activity of sinusoidal endothelium and a determination of any variation in phagocytic capacity. Other investigators (Bloom <sup>3</sup> and Higgins and Murphy <sup>4</sup>) have marked the Kupffer cells with particulate matter and subsequently observed the development of inflammatory lesions.

### METHOD

Granulation tissue was first produced and at intervals its endothelial cells tested for phagocytic capacity. Foci of granulation tissue in the liver of rats were produced in two ways. In one set of experiments a thick suspension of sterile casein was injected through a large needle with trochar lumen into the liver, and in a second series the liver margin was crushed with forceps. The vascularized lesions had much the same appearance except for the presence of numerous foreign body giant cells in the animals injected with casein. Also, accumulations of neutrophilic leucocytes appeared about the larger

\* Received for publication August 4, 1930.

casein deposits. In both sets of rats individuals were killed on the third, fifth, and seventh days. One to five hours preceding death, 0.5 cc. India ink was injected into the tail vein.

### DESCRIPTION OF EXPERIMENTAL LESIONS

In the sections from the animals killed on the third day after injection there are necrotic foci with disappearance of hepatic cell nuclei. The sinusoidal endothelial cells are here and there preserved, and the blood spaces which they line are continuous with the capillaries in the adjoining granulation tissue. Likewise, in some of the sections from animals killed on the fifth day, the necrotic cells are incompletely dissolved. Immediately about the necrotic lobules it is not always possible to determine whether the capillaries are persisting sinusoids or are capillaries newly formed in the granulation tissue. The position and direction of the capillaries in some of the locations show them to be new angioblastic tissue, and although mitoses are not numerous, several mitoses in interstitial cells may be seen in occasional single oil immersion fields. As regards carbon content, contrast of necrosis and granulation tissue foci with distant uninvolved portions of the liver is striking (Fig. 1). In the experimental lesions where the endothelium is no longer in contact with living hepatic cells there is relatively little phagocytosis (Fig. 2). In the rats killed on the seventh day, and in some at the end of five days, the hepatic cells are completely autolyzed. In these sections some collagenous material has appeared in the granulation tissue. In the intact lobules carbon is abundant, but phagocytosis is slight or absent where the sinusoids pass into the granulation tissue. This is true although the channels are obviously continuous.

### DISCUSSION

The question as to whether there are two distinct types of blood-vascular endothelium often is not clearly expressed in the discussions dealing with it. Maximow<sup>5</sup> has always stated his views definitely on this point. He believes that the sinusoidal (littoral, histiocytic) type is not true endothelium and has a separate embryonic origin: "The study of the embryonic histiogenesis of the connective tissue and blood shows, indeed, that the resting wandering cells (his-

tiocytes) of the embryo originate largely through the transformation of the lymphocytoid or histioid wandering cells into quiescent forms" (p. 177). He ascribes a dual origin, monocytic and histiocytic, to the migrating mononuclear phagocyte or polyblast. Most observers agree that the Kupffer cell is a normal integral part of the vascular lining of the liver. Eliot,<sup>6</sup> however, maintains that the histiocytes are merely monocytes temporarily anchored in the lumina of the capillaries of certain organs. That monocytes are present here in varying numbers is not disputed, but both the morphological and experimental evidence show that phagocytic cells are an essential part of the capillary walls. These permanently fixed phagocytes concern us in this discussion.

Do the Kupffer and other phagocytic cells that line the hepatic sinusoids differ from ordinary endothelium because of an unrelated embryonic origin according to Maximow's view, or is the difference the result of environment? The part played by the endothelium in metabolism is, in many particulars, ill understood. As emphasized by Aschoff,<sup>7</sup> it participates in lipid metabolism. In cholesteremia it undergoes profound structural change. In the larger blood vessels the function is at a minimum with slight passage outward of blood constituents. No doubt the function varies with the organ. In the lungs, with relatively little parenchyma, the transfer is chiefly gaseous and under normal conditions this endothelium shows slight phagocytic properties. In the liver, adrenal and pituitary, the endothelium is a bridge between blood and parenchyma and the liver especially is extremely active as regards phagocytosis. In the spleen and bone marrow, the cells that line the capillaries perform a normal phagocytic function especially concerned with the disposal of worn-out red corpuscles. Endothelium in these organs not only provides a smooth lining for the vascular network but also functions in the removal from the blood of particulate matter and colloids. In human pathology, and especially in destructive liver lesions, instances are not infrequently seen in which the hepatic endothelium in a new environment loses its phagocytic property. By the experimental destruction of the hepatic cells the capillaries that are newly formed, or those that represent persisting sinusoids, assume after a time the appearance of capillaries in granulation tissue elsewhere and lose much of their capacity for ingesting particulate matter. In the normal liver as the capillaries leave the periportal tissue to penetrate the

lobule the endothelium establishes contact with the hepatic cells and assumes an added function. When this function is lost, the cells revert to the usual inactive type.

### SUMMARY AND CONCLUSIONS

1. In granulation tissue within the liver, the capillaries may arise in the usual way by angioblastic proliferation, or sinusoids may persist as capillaries. In both instances the vessels are continuous with the sinusoids in the adjoining lobules.

2. With loss of contact with living hepatic epithelium the endothelium becomes less phagocytic.

3. The peculiarities of sinusoidal endothelium depend on its functional activity, which, in turn, is determined by its normal histological position. From the behavior of sinusoidal endothelium present in granulation tissue, it appears not to be a distinctive type of cell.

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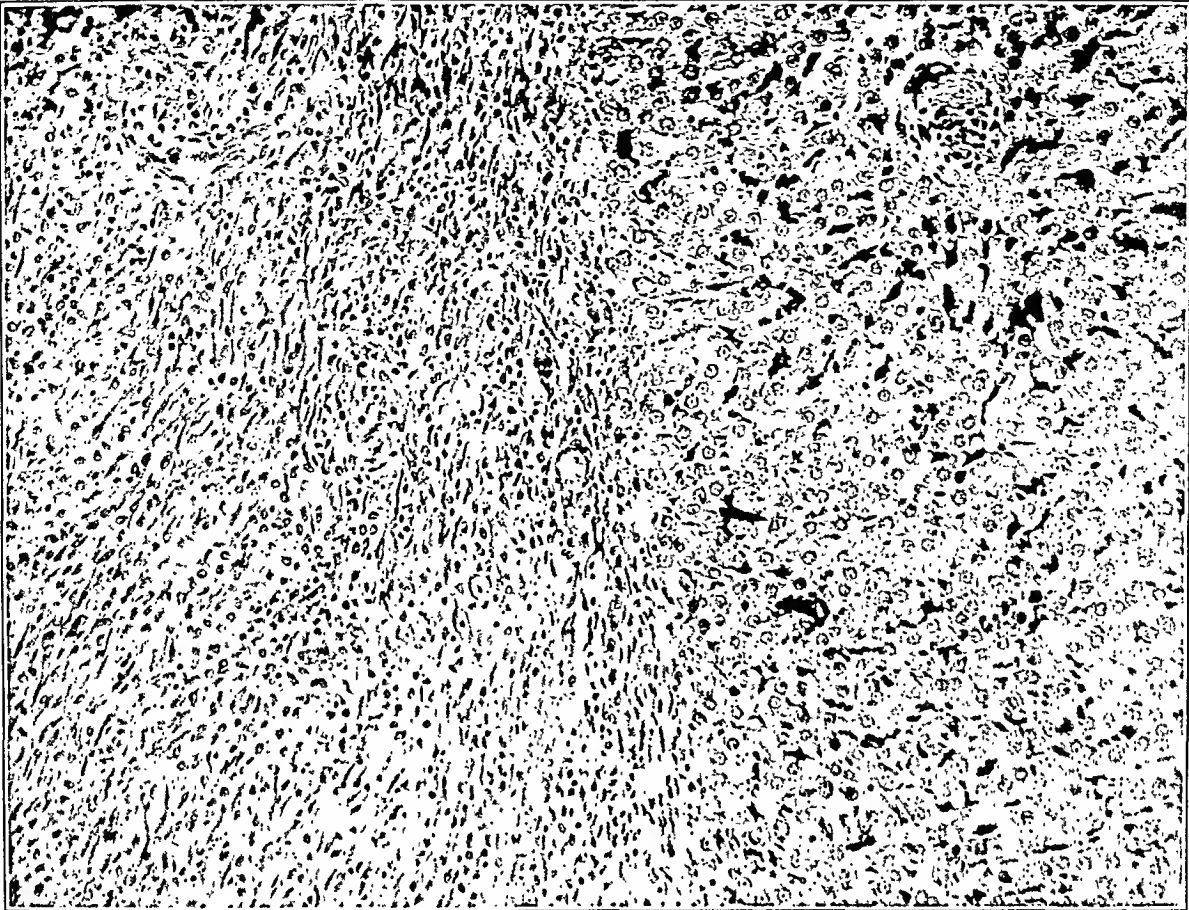
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### DESCRIPTION OF PLATE

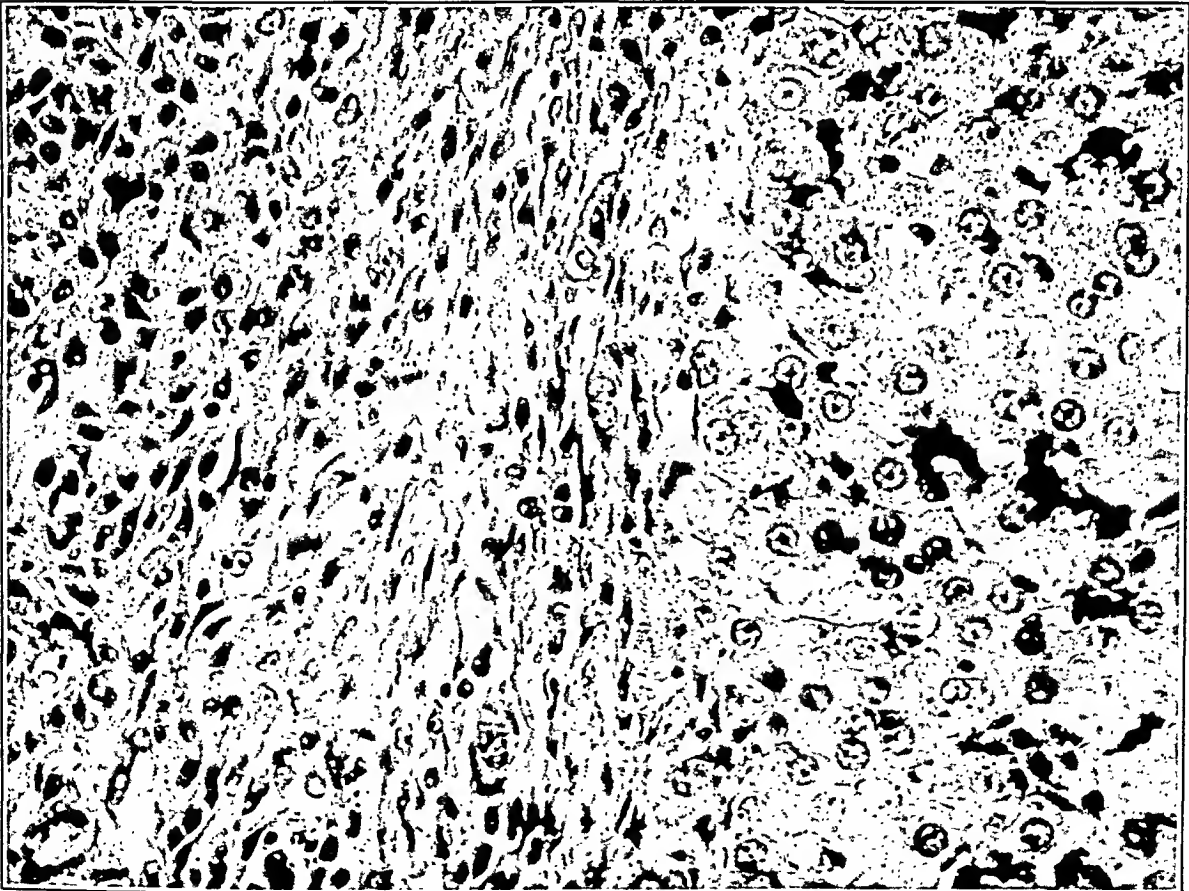
#### PLATE 2

FIG. 1. Sinusoidal endothelium within liver lobules packed with ink particles. The capillary endothelium of the highly vascularized granulation tissue is practically devoid of carbon. Five-day granulation tissue in liver of white rat.  $\times 200$ .

FIG. 2. Endothelial cells of the sinusoids with huge amounts of ingested carbon. Endothelium of abundant capillaries of the granulation tissue shows little phagocytosis. In granulation tissue in the liver the phagocytic activity on the part of the vascular endothelium is no greater than that of granulation tissue in general that has not been subjected to artificial stimulation. Granulation tissue five days old.  $\times 500$ .



1



2



## BENIGN POLYMORPHOCELLULAR TUMORS OF THE KNEE REGION \*

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The somewhat variable, but specific structure and benign clinical course of a group of slowly growing tumors arising from tendon sheaths and aponeuroses, commonly in the hands, much less frequently in the feet, and very rarely in other locations such as the knee, have been recognized since the first descriptions of the growths by Gross,<sup>1</sup> Paquet,<sup>2</sup> Reverdin,<sup>3</sup> and Heurtaux.<sup>4</sup> These early French writers were much impressed by the presence in the tumors of large giant cells ("myeloplaxes"), resembling those of epulis and other benign bone neoplasms. Heurtaux distinguished the newly described tumors from sarcoma by the clinical results in the cases and termed them "myelomas." This name although obviously a misnomer, has persisted almost to the present day. Such tumors are comparatively rare, as evidenced by the fact that Spiess<sup>4</sup> was able to collect only forty-five instances from the literature up to 1913. The peculiar complex and puzzling structure which characterizes the newgrowths has ever been a matter of considerable interest to clinicians and pathologists in Europe, particularly in France and Germany, so that a fairly voluminous literature on the subject has grown up in the languages of these countries, while comparatively little is to be found in English.

Almost from the beginning there has been controversy as to the nature and pathogenesis of the tumors. Some writers, beginning with Fleissig<sup>5</sup> in 1913, have contended that these peculiar tumors are inflammatory granulation growths although others have always regarded them as true, but benign neoplasms. Lecène and Moulonguet<sup>6</sup> compare the tumors to changes resulting from chronic irritation of long duration, such as occur in old hematocele, hernial sacs and chronic bursitis. Talbot, who cites these two authors, speaking of tumors with myeloplaxes occurring in the articular

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Although rarely the site of primary tumor formation, the capsules and synovial membranes of joints give rise to a variety of neoplasms including hemangioma, lipoma simplex or arborescens, chondroma, osteoma, malignant sarcomas, and finally the so-called xanthomatous giant cell tumors. Hartman<sup>15</sup> was able to collect only sixteen examples of capsular and synovial tumors from the literature prior to 1922, to which he added another. The knee was the seat of involvement in fifteen of the seventeen cases. Garrett<sup>16</sup> reports four instances and Harbitz<sup>17</sup> eleven from his own material, ten of which occurred in the knee while the other involved the wrist. Although some one hundred and twenty cases of tendon sheath and synovial membrane tumors had been recorded in the literature prior to 1922, Hartman<sup>15</sup> discovered that in spite of exhaustive study no one had previously compared or identified the tumors arising from the synovial membranes of tendon sheaths and those primary in the synovia of joints. He distinguishes three groups of synovial tumors: (1) pedunculated tumors; (2) growths with giant cells, and (3) diffuse tumors without giant cells. He would class the growths with the benign tumors of connective tissue origin, although there is a potential malignancy which may be enhanced by palliative measures and incomplete excision. He feels that these neoplasms should not be termed sarcoma unless clinical or pathological evidence of malignancy appears. By reason of the fact that any one of the characteristic cells listed by Ewing may be absent from an otherwise typical case, Hartman prefers the name of myeloid tumor above others.

A search through the available references since Hartman's review in 1922 has shown the need for bringing the literature concerned with xanthomatous giant cell tumors of the knee joint up to date, and this will be attempted as briefly as possible. Instances of frankly malignant sarcoma, as well as benign neoplasms other than the type mentioned above will be omitted.

#### SUMMARY OF CASES

Garrett's study,<sup>16</sup> which appeared two years after Hartman's publication, seems to have been overlooked by recent European writers.

Two of Garrett's cases are without clinical records and only the pathological diagnosis is given. One showed foam cells, capillary

angioma, giant cells and pigment, the other was called a giant cell fibrohemangioma or xanthoma. The third example was that reported by Hartman. Case 4 was a woman, aged 21 years, who had a painful and swollen knee for six months prior to operation. Necrotic tissue was found below the patella and the affected substance was removed. Microscopic examination disclosed numerous giant cells at first suggesting giant cell tumor of bone, but the cells were embedded in a different type of tissue, were smaller and spindle-shaped. Endothelial-lined blood spaces were present. There were no foam cells. Although the growth was not removed completely the patient was well six years later.

In Cabot's Case Records <sup>18</sup> in 1926 appears an account of a tumor of known etiology, in some respects simulating the type under consideration. A male 24 years of age, suffering from acute infectious arthritis of the knee was at first treated by injection of 2 per cent formalin in glycerin. Arthrotomy with removal of a tumor three years later disclosed a growth closely associated with the entire synovial membrane, of rough fibrous consistency with roughening and erosion of bone and cartilage. Microscopically the structure consisted of small mononuclear wandering cells with newly formed blood vessels, a fibrous stroma forming an intercellular substance in which single cells were embedded. A previous biopsy section had disclosed numerous foreign body giant cells. The histological appearance bore some resemblance to a giant cell tumor. The growth was considered a chronic inflammatory process.

Harbitz <sup>17</sup> in 1927 listed the following nine cases of tumors originating in the articular capsule and synovial membrane of the knee or adjacent areas.

*Case 1.* In a woman, aged 51, a tumor, the size of a pigeon's egg, developed in the capsule of the knee in the course of two years. It was firm, gray-white, fibrous, not distinctly limited and microscopically a polymorphocell sarcoma, with giant cells in some places, and containing some pigment.

*Case 2.* In a woman, aged 66, a tumor, the size of a walnut, was removed from the capsule of the knee joint. It was firm and hard, with a gray-white, cut surface; microscopically, it proved to be a small cell sarcoma. In the following four years it did not recur.

*Case 3.* A woman, aged 27, for some years had had a swelling in the recessus superior genu, which microscopically proved to be a flat, firm, fibrous, gray-white tumor, a sarcoma fusocellulare.

*Case 4.* Ostensibly after an injury in a man, aged 26, a peduncular tumor the size of an almond developed in the synovial membrane of the knee joint, a sarcoma fuso-giganto-cellulare, microscopically.

*Case 5.* Ostensibly after an injury to the knee, small tumors developed on the outer side of the capsule in a woman, aged 39. Microscopically this appeared to be a polymorphocell sarcoma.

*Case 6.* A patient had a recurrent polymorphocell sarcoma on the inner side of the knee joint capsule. Death resulted from return of the growth.

*Case 7.* In a woman, aged 71, a large flat sarcoma, fibro-giganto-cellulare, formed in the capsule of the knee joint in the course of five months.

*Case 8.* In a man, aged 22, a sensitive neurofibroma, the size of a bean, developed in the capsule of the knee joint in the course of two years.

*Case 9.* A sailor, aged 58, presented a calcareous fibroma, the size of a goose egg, which had existed for thirty years on the inner side of the knee."

Harbitz believes these growths form a special and separate group, less well defined than those originating from tendon sheaths, but connected with them by transitions. He regards the structure of articular capsule and synovial newgrowths as more purely sarcomatous than those derived from the sheaths of tendons, containing fewer giant cells, little pigment and no xanthomatous tissue. In my opinion Cases 8 and 9 do not properly belong in the group of capsular and synovial tumors. In addition to the neoplasms already mentioned, Harbitz describes another, the clinical history of which was furnished by Dr. Sundt.\* The growth occurred in a young woman 20 years of age, whose right knee became swollen after a blow. A year later a cast was applied for supposed tuberculosis, without benefit. On opening the joint a little bloody fluid, a thickening and reddish brown discoloration of the capsule were noted. There was some thinning and honeycombing of the articular surface of the femur. The microscopic pathological diagnosis was probable xanthofibrosarcoma gigantocellulare pigmentosum. Signs of inflammation were lacking and nothing suggested the organization of extravasated blood or exudation. The tumor much resembled the xanthosarcomas of tendon sheaths and like them appeared comparatively benign, although giving some evidence of destruction of cartilage and bone.

Abadie<sup>19</sup> in 1928 reported two rare tumors of the knee, one primary in the synovial membrane, the other a patellar bone tumor diagnosed "tumeur à myeloplaxes like certain types of epulis." Only the first will be mentioned. A woman 45 years of age, had for several months noted a small mass on the external surface of the right knee. Operation revealed a small discoid growth attached to the synovial

\* Sundt, H. (*Norsk. mag. f. lægevidensk.*, 1929, 90, 521) has redescribed this case in detail, together with an instance of chondromatosis of the knee. He states that the woman was well four years after the operation.

membrane, necessitating excision of a small portion of it. The growth was of leather-like consistency, café au lait colored, with some dark yellow patches. Histological examination showed a type of granuloma with a predominance of typical lymphocytes, but also including multinuclear giant cells and ochre-colored pigment both free and within macrophages.

The tumor described by Talbot<sup>6</sup> occurred in the left ankle of a man, 20 years of age. Following a fracture and refracture four years before, treated by traction at the ankle, a swelling appeared. Operation disclosed an irregular tumor 5 by 3 by 2 cm. lying partly in the outer plane of the capsule but arising mostly from the inner surface of the synovial membrane. The color varied from saffron yellow to ochre. The conclusion reached after microscopic study was: "a connective tissue tumor, a granuloma with giant cells which microscopically would have been called sarcoma a few years ago." Iron-containing pigment was present also. Improvement was noted.

Talbot describes in some detail the case of Paitre and Bruas (*Soc. de méd. mil. franç., Bull. mens., Nov., 1923*).

A cavalryman had injured his left knee in a fall one year before with only temporary impairment of function. A new fall brought him to Paitre and Bruas who found a localized swelling the size of an apricot pit which seemed to enucleate itself from the depths of the articulation and appeared to be situated on the patellar ligament and inseparable from it. A tumor the size of a mandarin orange was enucleated. The mass was implanted with the fat pad in the bottom of the intercondylar notch. A good functional result followed the operation. The pathological diagnosis was: "fibrosarcoma with myeloplaxes, tumor histologically benign."

Wegelin<sup>8</sup> has seen one instance of benign xanthomatous giant cell tumor in a 43 year-old man, who for eight years had noticed a cyst on his knee clinically having the appearance of a joint-mouse. At operation a pedunculated mass was found and excised. The pedicle of the growth was attached to the joint capsule beneath the patella; the mass was lobulated, firm, grayish red, in places yellowish, and measured 3.5 by 2 by 0.5 cm. Sections exhibited a richly cellular growth, for the most part spindle-shaped and with abundant cytoplasm. Irregularly distributed throughout were numerous multinucleated giant cells. A fine collagenous intercellular substance separated the tumor cells. Extra- and intracellular brownish pig-

ment, large and small groups of fatty cells containing little doubly refractive substance, and clefts covered by endothelial-like, cubical or low cylindrical cells completed the picture. There was no recurrence after ten years.

Wegelin's second case was a complex structure of distinctly sarcomatous nature which he believes may have been the result of a differentiation of cells from a newgrowth of the xanthomatous type.

Other cases mentioned by Wegelin follow:

Weil (*Berl. klin. Wchschr.*, 1915, 52, pt. 1, nr. 6, 129), and Seyler (*Virchows Arch. f. path. Anat.*, 1922, 239, 20) each mention xanthomatous tumors of the joint capsules, but their descriptions as to the exact location of the growths are not clear.

Züllig (*Cor.-Bl. f. Schweiz. Ärzte*, 1917, 47, 1368). A woman 34 years of age, complained of pain and marked effusion in the right knee without previous trauma. Several pedunculated tumors which extended into the joint cavity were removed. Microscopically these were found to consist of spindle, polymorphous and giant cells with rich deposits of cholesterin in many places imparting a xanthomatous appearance. Recurrence had not taken place one year later.

Wustmann (*Deutsche Ztschr. f. Chir.*, 1925, 192, 381). For several years following an injury of the left knee a woman 42 years of age had what appeared clinically to be a joint-mouse. At the operation a polypoid, polycystic, chestnut-sized tumor, microscopically exhibiting many blood vessels, xanthoma cells and giant cells was found. Wustmann concluded that the growth was derived from the vascular endothelium.

Herrheimer (*Arch. per le sc. med.*, 1927, 50, 201, Festschr. f. Morpurgo) mentions a yellowish proliferative villous synovial tumor of the knee joint in a woman of 30 years. Spindle cells, giant cells and xanthoma cells were observed in microscopic preparations.

Frangenheim's paper<sup>7</sup> is based upon observations in a man of 50 years. Nineteen years before, the left knee had been dislocated, followed by effusion and later subsidence of symptoms. After nineteen years a swelling appeared, accompanied by very severe pain. A firm brownish mass the size of a hen's egg, in part broadly attached to the synovial membrane, and partly pedunculated, was removed surgically. Histological examination by Professor Dietrich showed a tumor composed of round and polygonal cells separated by a fine fibrillar stroma, numerous giant cells, blood and pigment deposits,

and having much the appearance of a giant cell sarcoma of the epulis type.

Five years later, and six months before coming to Frangenheim's clinic, the growth reappeared, accompanied by pain and effusion from the medial surface of the knee and popliteal space. On incising at the site of the previous operation, bloody fluid escaped from the joint cavity. The entire synovial membrane was covered with brownish villi of varying lengths. It was necessary to remove the anterior part of the joint capsule. A hygroma lying in the popliteal space and lined by the same villous structures as the growth in the knee was excised at the same time.

Professor Siegmund, who studied the sections, reported a new-growth of granulation tissue type, richly vascular, distinguished by the presence of giant cells, the formation of a homogeneous osteoid intercellular substance, groups of iron-containing cells and lipid. He regarded the growth as a benign resorption tumor belonging in the class of so-called giant cell sarcoma of tendon sheaths. Six months later the patient had no symptoms referable to the knee. Frangenheim states that the presence of osteoid intercellular substance has never before been observed in this type of tumor and that such a finding makes the similarity of structure to that of epulis still greater.

Frangenheim cites the following authors not mentioned by Wegelein.

Tobler (*Beitr. z. klin. Chir.*, 1927, 140, 545). The tumor arose from the medial meniscus, was cherry-sized, flat, yellowish red, in places brownish and had a thin pedicle 3 mm. long originating from the joint capsule.

Albertini (*Gutartige Riesenzellgeschwulste*, G. Thieme, Leipzig, 1928) without clinical data, mentions a free joint body of the knee which he called a xanthosarcoma.

Simons (*Neue deutsche Chirurgie*, 1928, 43, F. Enke, Stuttgart). A man of 50 years stated that six months before his right knee had been injured in a fall against a sharp edge. For the last six weeks the pain had been severe and lightning-like, accompanied by swelling of the joint. A firm body could be felt, disappearing on flexion and re-appearing on extension. A disc-shaped mass, the origin of which was difficult to determine and at first thought to be a meniscus, was discovered at the operation. Histologically it consisted of xanthoma

cells and a sarcomatous-like basal structure. Pathological diagnosis: xanthosarcoma.

Négric and Canton (*Bull. et mém. Soc. nat. de chir.*, 1929, 55, 617). A male, 20 years of age, was admitted to the hospital with a diagnosis of articular foreign body in the right knee. Three years previously he had suffered a severe injury of this knee, necessitating bed rest for three months. One year later the knee would swell suddenly on the slightest traumatism. A voluminous, hard and easily movable object giving the impression of a foreign body could be felt along the medial margin of the knee. Flexion caused it to disappear. Arthrotomy disclosed a growth at the level of the pre-spinal surface of the tibia and adherent to the synovial membrane. The object removed looked like a pebble, slightly excavated on one side, protuberant on the other, and measured 4 by 3 by 2 cm. Microscopic examination revealed a myeloplaxoma having a framework of connective tissue showing a discrete inflammatory reaction, traces of blood pigment in the process of resorption and large multinuclear giant cells. The patient was discharged as cured after six months.

These two authors saw two cases but the other is not described. They regarded the growth as benign and the result of an inflammatory reaction rather than a tumor in the true sense of the word.

### CASE REPORTS

The material here presented was obtained from a father and his daughter both of whom were operated upon by Dr. Harry C. Blair. A clinical study is being prepared by Dr. Blair and will be published separately.

CASE I. Mrs. H. E. P. (29272), age 31 years. The patient was entirely well until a year ago when her right knee was injured by striking it against a bench. Sudden and severe pain followed by swelling forced her to remain in bed for a week. Thereafter the knee appeared normal for a time but six months ago a gradually increasing swelling appeared above the patella. The knee never catches, movement is not limited, pain is slight and absent at night. No other joints are involved.

Inspection revealed no apparent enlargement of the affected knee but on palpation a small mass which seemed to roll beneath the vastus medialis muscle was made out. The movements of flexion and extension were normal. There seemed to be a small amount of fluid within the joint cavity. Roentgenogram was negative. A blood cholesterol estimation was not done.

The patient consented to operation. An incision along the medial aspect of the thigh over the region of the quadriceps bursa exposed a tumor mass which was found to make up a portion of this bursa but lay mostly within the main articular cavity of the knee. The intra-articular portion had a peculiar "medusa-head" appearance. The growth was removed without difficulty, the edges of the capsule were coapted, the postoperative course was uneventful and the patient was walking without aid after a month. Eight months later she was still well.

*Gross Examination of Specimen:* The tissue consists of a very ragged soft appearing but firm mass measuring 12 by 5 by 2 cm. in its maximum diameters, and weighing 35 gm. Along one border is a thin layer of adipose tissue, and adjacent to this is a shiny, yellowish white membranous area, possibly representing a portion of synovial membrane, but in the main the growth is made up of a fairly firm faintly yellowish to yellowish white substance devoid of visible blood vessels. Extending irregularly through the tumor are many wavy, light yellowish brown to brownish streaks producing a somewhat laminated effect. Extending outward from one border are numerous thread- and band-like extensions, some only a fraction of a millimeter in diameter, others as much as 5 mm. in thickness. For the most part these form bridges between the main tumor and another similar but smaller mass, but some have one free end. The longest band is formed by the union of two smaller ones and on its opposite end there is suspended an irregularly outlined brownish structure identical in appearance with the main growth.

*Microscopic Examination:* Sections from different parts of the specimen reveal a peculiar histological structure. In the main, the growth seems to be made up of numerous closely packed cells of variable size and shape having a faintly reticular, foamy-appearing and abundant cytoplasm. Some are round or oval, others polygonal or spindle-shaped. Their nuclei are on the whole rather small, pyknotic and generally centrally situated. The growth is much more vascular than the gross appearance would indicate. Scattered throughout are numerous small and collapsed, or large and distended capillary blood vessels, together with occasional arterioles. Three distinct changes occur about the blood vessels; in some instances these consist of radially arranged collars of small oval-shaped cells having hyperchromatic nuclei; others are surrounded by irregular zones of phagocytic cells laden with both finely and coarsely granular yellowish brown pigment giving a strong Prussian blue reaction;



still others are collared by lymphocytes. These areas correspond to the softened brownish zones observed macroscopically. Occasional multinuclear giant cells are found in the vicinity of the pigmented areas and blood vessels, but are nowhere numerous and in the fatty cell areas are absent altogether. The giant cells vary considerably as to size and the number of nuclei within them; their nuclei are closely packed, lie toward the central part of the cells and in many respects resemble closely those found in benign giant cell tumors of bone. Hemosiderin pigment granules occur in some of these cells. None of the various types of cells show mitosis of their nuclei and nothing suggestive of malignancy is observed. No bursal structure is recognized microscopically.

Sections stained for fat with Sudan III reveal an abundance of small and medium sized bright orange-colored droplets within the cytoplasm of the foamy appearing cells throughout the growth. The pigment-containing cells and giant cells appear dark brownish in these preparations.

*Pathological Diagnosis:* Benign polymorphocellular tumor arising from synovial membrane of right quadriceps bursa and extending into the main articular cavity of the knee.

CASE 2. Mr. J. S. (30377), age 68 years, father of Mrs. H. E. P., presented himself, walking with the aid of a cane and scarcely limping. He stated that he had worked every day of his life and had always enjoyed good health. Forty-five years ago, while a cadet in an officers' training school in Russia, a horse had kicked his left knee. The resulting disability caused his retirement from the Army and he emigrated to America. After the injury the knee remained swollen and enlarged slowly. During the past three years there has been a more rapid increase in size, especially during the last three months.

The left knee was seen to be greatly enlarged, measuring 90 cm. in circumference, as compared to 39 cm. on the unaffected side. Many large veins were visible under the skin about the knee. Extension was complete and flexion possible to 45 degrees. The foot on the affected side was swollen and edematous. The dorsalis pedis and posterior tibial arteries were palpable.

Amputation was deemed the operation of choice. The patient refused but consented to an exploration and biopsy. A medial incision revealed a large encapsulated mass lying beneath the thinned-out vastus medialis muscle. Bleeding was profuse but sufficient tissue was obtained for pathological examination. An uneventful postoperative course followed, complicated only by the development of a symmetrical edema extending from the umbilicus caudally. Consent for amputation was now obtained and the leg was severed proximal to the tumor mass. The edema disappeared entirely within twenty-four hours and did not recur. The wound healed without infection and after four months the patient was able to walk with the aid of an artificial limb. At the time of writing, seven months after the amputation, there has been no local recurrence or signs of metastases.

*Gross Examination:* The left leg has been amputated at the junction of the middle and distal thirds of the femur. The knee is enormously increased in size, rounded and lacks the normal contour and anatomical landmarks. The circumference is 70 cm. at the knee, 48 cm. over the calf and 29 cm. at the ankle. There is a marked pitting of the skin on pressure over the entire foot and leg. The skin over the lower third of the leg is dark brownish and scaly but elsewhere is whitish. On the medial surface of the knee is a long, recently healed surgical wound with a reddish scar.

On sagittal section through the femur, knee joint and tibia the subcutaneous fat, muscles and fascia appear to be very edematous. The veins are filled with soft blackish non-adherent clots. The popliteal artery is displaced far posterior to its normal position by a large tumor mass, but its lumen is patent. Entirely surrounding the knee joint and extending for a distance of 25 cm. superiorly, and 20 cm. inferior to it is an enormous, irregularly lobulated growth. The largest single tumor has a maximum diameter of 11 cm. and is situated posteriorly in and above the popliteal space. This mass involves the posterior capsule of the knee joint, extends upward along the periosteum of the femur, then posteriorly into the popliteal space. It is quite irregular in shape but the peripheral border is sharply outlined. The color varies from light yellowish to a rusty brown with the two colors often intermingled. The tissue has a honeycombed appearance in places while other areas are solid and firm. A sticky yellowish substance exudes from the cut surfaces. Situated below the knee and posterior to the tibia are several similar but smaller nodules, one of which has invaded the capsule of the knee. On the anterior surface of the thigh and directly invading and destroying the capsule of the knee joint is another large mass somewhat similar to the one first described, except for the color, which is almost uniformly a deep rusty brown. The knee joint is filled with a soft brownish substance which covers and obscures the medial meniscus. The lateral meniscus is unchanged. In places the cartilage over the femoral condyles is thinned but not entirely eroded. The same is true for the head of the tibia. A somewhat curved area of erosion is observed in the posterior cortex of the patella. The bed of this defect is filled with a brownish substance. Similar-appearing material fills the recessus superior. The growth stops short just below the head of the tibia in the sagittal cut but is found further out in the

soft tissues for some little distance below this point. At the point of attachment of the anterior cruciate ligament to the femur, there is a soft dark brownish area with a solid yellowish border extending for a distance of 1.5 cm. through the synovial membrane and cartilage into the marrow of the femur, where it ends abruptly.

*Microscopic Examination:* In sections stained with hematoxylin and eosin the character and appearance of the growth are found to vary with the area from which the blocks were selected. Those from the faintly yellowish white zones show rounded or oval cells of rather uniform size and containing small and somewhat pyknotic nuclei. The cytoplasm has a distinct foamy appearance and stains only faintly with eosin. In portions of such areas the cells have undergone partial or complete necrosis. In the brownish zones some of the cells are of the same type as those just described, but the discoloration is undoubtedly due to the presence of a large amount of coarsely granular yellowish brown pigment particles occurring intracellularly for the most part. The pigment is often so abundant as to fill the entire cell cytoplasm, but in some instances the foamy appearing cells contain only a few granules. Other parts of the tumor exhibit a still different picture in that the cells are stellate, polygonal or spindle-like and the cytoplasm is homogeneous and stains well with eosin. The nuclei too are larger, rounded or oval and tend to be hyperchromatic, often with one or two small nucleoli, but exhibit no mitoses. In places the cells are compact but more generally are somewhat separated by clear spaces. Careful search reveals a few large multinucleated giant cells of the foreign body type. Lying here and there in the growth are strands of fascia, fat cells, and small blood vessels which show no tumor cells within their lumina. At the periphery the newgrowth is sharply demarcated from the atrophic muscle and connective tissue cells surrounding it. In sections stained with ferrocyanide-carmin the brownish pigment appears bluish, proving the presence of iron. Sudan III preparations disclose an abundance of varying sized globoid masses in the foam-like cells. In some instances the color is bright orange, in others the substance has a dirty orange-brown hue. Stains for mucin give a negative reaction and Van Gieson's preparations disclose relatively little supportive tissue within the newgrowth. A block of bone taken from a point near the insertion of the anterior cruciate ligament where macroscopic evidence of invasion by the newgrowth is apparent, reveals

many pigment-filled tumor cells lying in the marrow, but even here the line of demarcation is quite sharp and the cells are not more anaplastic than elsewhere in the growth.

*Pathological Diagnoses:* Large benign polymorphocellular tumor of left knee invading the joint capsule, femur and patella; recently healed surgical wound; healed varicose ulcer of leg with pigmentation of skin; marked edema of foot and leg.

## DISCUSSION

The location, macroscopic appearance and histological structure of both tumors herein described conform in every respect to that of the poorly understood, variously termed, but nevertheless definitely recognized group of newgrowths arising usually from the sheaths of tendons and aponeuroses, but also rarely from joint capsules and synovial membranes. Histologically the two growths are identical. Each is richly vascular, contains xanthoma-like cells filled with both unsaturated fat staining well with Sudan III, or a muddy yellowish brown-colored material which is probably cholesterol, multinucleated giant cells largely of the epulis variety, abundant extra- and intra-cellular pigment giving a positive reaction for iron, and other cells of the most varied morphology.

In Case 1 the growth was comparatively small and its origin was established definitely as coming from that portion of the synovial membrane of the knee joint forming the quadriceps bursa. From here many irregular villous projections extended into the articular cavity proper. The tumor therefore belongs in the pedunculated group of Hartman's classification. The relatively slow growth, comparatively small size of the mass, lack of bone involvement or demonstrable metastatic deposits either prior to, or since the operation, together with the innocent microscopic appearance indicate an essentially benign character.

The newgrowth in Case 2 is most remarkable by reason of the extremely long clinical course of forty-five years and because of its enormous bulk. The latter feature makes this tumor quite unique among others of its kind since it is apparently by far the largest ever reported. Few of the tumors recorded in the literature have been larger than a hen's egg even though present for many years. On account of the great size and extensive ramifications of the growth, it was quite impossible to determine the original point of departure.

However, since the bulk of the mass lay in the soft tissues about the knee with comparatively little tumor tissue within the joint cavity, it is reasonable to assume an extra-articular origin, possibly from one of the tendon sheaths about the knee. In spite of the bulkiness of the tumor, the history of recent rapid enlargement and the obvious evidence of penetration of the joint capsule and destruction of bone, there is no microscopic evidence of a malignant departure. The lack of demonstrable metastases before or after amputation of the leg is further evidence of its benign nature. In the presence of a growth of such generous dimensions one is not surprised to find some destruction of soft tissues and even bone, either as a result of pressure atrophy, repeated hemorrhages, interference with the blood supply or from a combination of one or all these factors. If it were possible to state with certainty that the tumor in Case 2 had an extra-articular origin the present study would offer still further evidence of the now generally recognized fact first pointed out by Hartman that identical newgrowths may arise from the sheaths of tendons and synovia of joints. Unfortunately, for reasons mentioned previously, such proof is lacking in the second case.

The occurrence of identical newgrowths in the same location in closely related individuals is interesting but may be only a coincidence.

Etiologically, trauma appears to have been the immediate exciting cause for tumor formation in both individuals. Although a history of injury is lacking in many of the previously recorded instances of these tumors, it appears to have been definitely associated with the onset of the growths in so many others that one cannot escape the conviction that trauma may play an important etiological rôle.

In the more recent European literature on xanthomatous giant cell tumors of joints there is a distinct tendency to regard the growths as granulomatous lesions rather than true neoplasms. On this point the writer has little to offer except that it is difficult to believe that a granuloma could attain the size of the growth in Case 2. The fact that apparent transition from such growths to frankly malignant sarcoma occurs, as pointed out by Harbitz and Wegelin, is likewise evidence of their being neoplastic rather than granulomatous.

The problem of nomenclature on the basis of cell morphology is a troublesome one because of the variety and variability of component cells in individual cases. Although most of the growths contain at

least some of the cell types mentioned by Ewing, others do not. The tendency has always been to name the tumors according to the most striking characteristic or characteristics, with the result that none of the terms is wholly inclusive or completely satisfactory. The recent tendency on the part of nearly all writers to drop the term sarcoma and to condemn its use is a distinct advance. Hartman's view that such tumors should be classed as benign growths until clinical or pathological evidence of malignancy is manifested is logical and sound. The writer has therefore refrained from calling the two tumors reported in this paper sarcomas. The designation "polymorphocellular tumor" introduced by Harbitz is less misleading and more descriptive than any of the many names by which the tumors have been known in the past, and I have therefore adopted it in preference to others.

#### SUMMARY

1. Two instances of benign polymorphocellular tumors occurring in the knee region of closely related persons are described.
2. In the daughter, the newgrowth originated in the synovial membrane, a rare site of tumor formation, and was benign.
3. In the father, the tumor was of exceptionally long duration, attained very bulky dimensions and although exhibiting locally destructive properties also appears to have been benign. Because of the large size and extensive ramifications of the mass, the point of origin could not be determined, but certain facts point to an extra-articular source.
4. Most commonly newgrowths of the type herein reported arise from the sheaths of tendons and only rarely from the capsule or synovial membrane of joints. In the latter event the knee is much more frequently involved than any other joint in the body. The rarity of such joint growths is evidenced by the fact that the author was able to collect from the literature, in addition to his own cases, only forty-four instances of closely similar or identical tumors.

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## DESCRIPTION OF PLATES

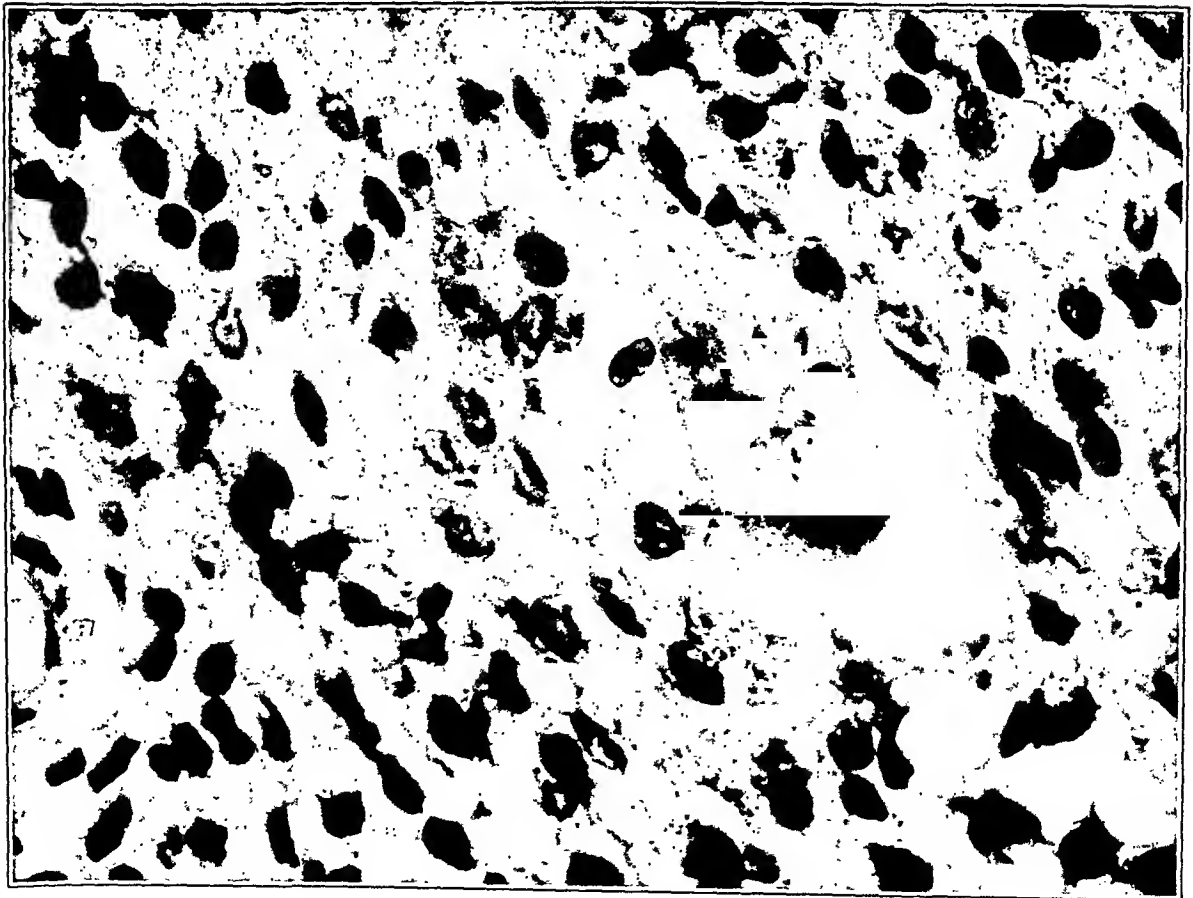
### PLATE 3

FIG. 1. Case 1. Photograph of tumor removed from quadriceps bursa and extending into articular cavity of knee. Right: ragged external surface with dependent mass connected with main tumor by a thread-like band. Left: cross-section of growth showing pale xanthomatous areas separated by brownish pigmented zones.

FIG. 2. Case 1. Medium high power photomicrograph illustrating the polymorphocellular character of the tumor. The fine blackish intracellular bodies are hemosiderin pigment. A multinucleated giant cell and capillary blood vessel are included in the field.



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Hunter

Benign Polymorphocellular Tumors of Knee Region



#### PLATE 4

FIG. 3. Case 1. High power view from area adjacent to that shown in Fig. 2. Scattered throughout are enormous foamy-appearing cells distended with lipoid. The irregular blackish masses are aggregations of hemosiderin. Several spindle cells occur in the upper center. A single capillary may be seen in the lower left corner.

FIG. 4. Case 2. Photograph. Sagittal cut through left knee showing the macroscopic appearance and location of the newgrowth, the character of which is well shown in the large mass situated in and proximal to the popliteal space. The center is rusty brown and honeycombed, the periphery nearest the femur yellowish white, solid and xanthomatous. The very dark tumors contain a great deal of hemosiderin throughout. Destruction of the joint capsule is evident both posteriorly and anteriorly. In sawing the specimen a part of the medial condyle of the femur was torn away giving the appearance of far more bone destruction than actually existed. The medial meniscus is covered with brownish tumor substance which also fills the recessus superior. An irregular area of erosion is visible in the posterior cortex of the patella.



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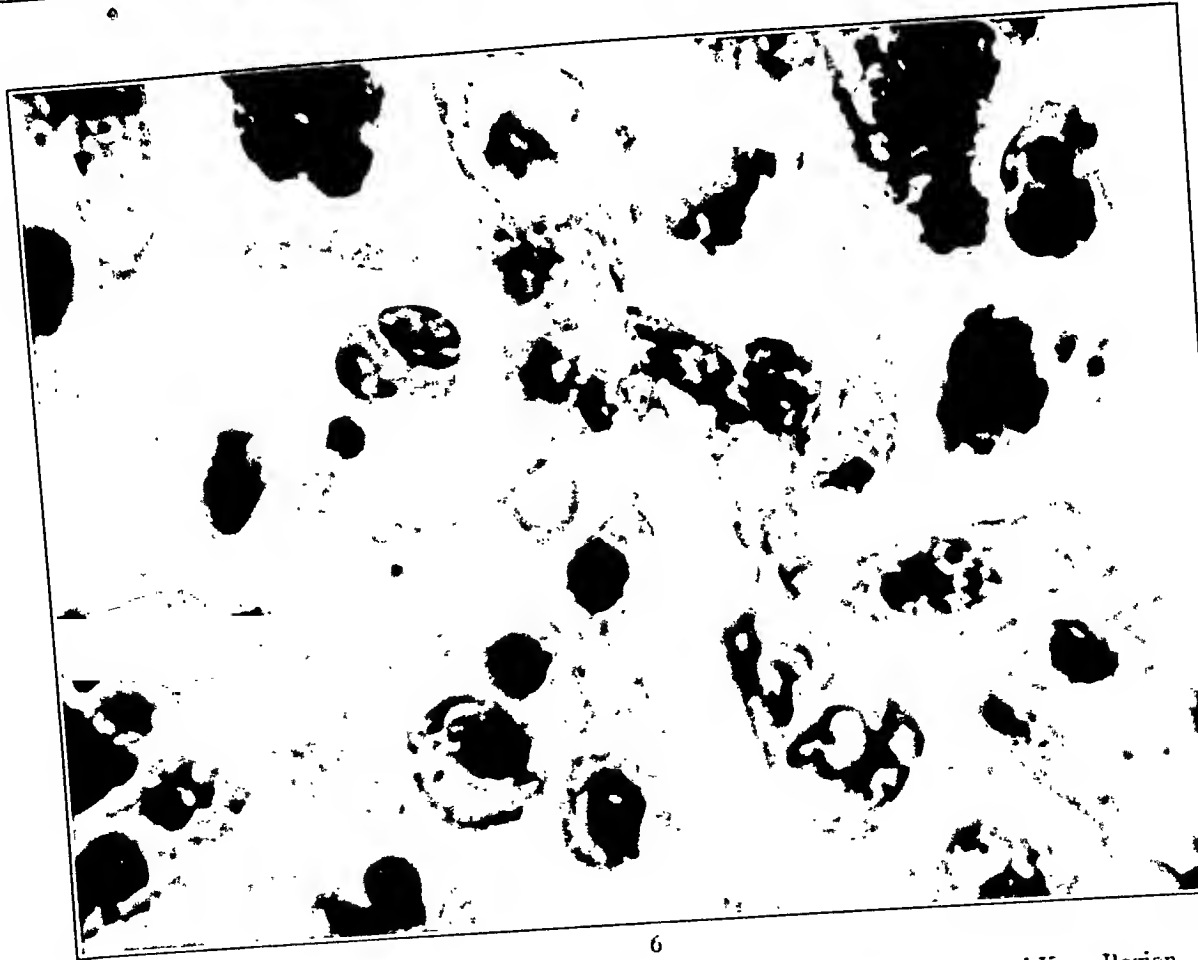
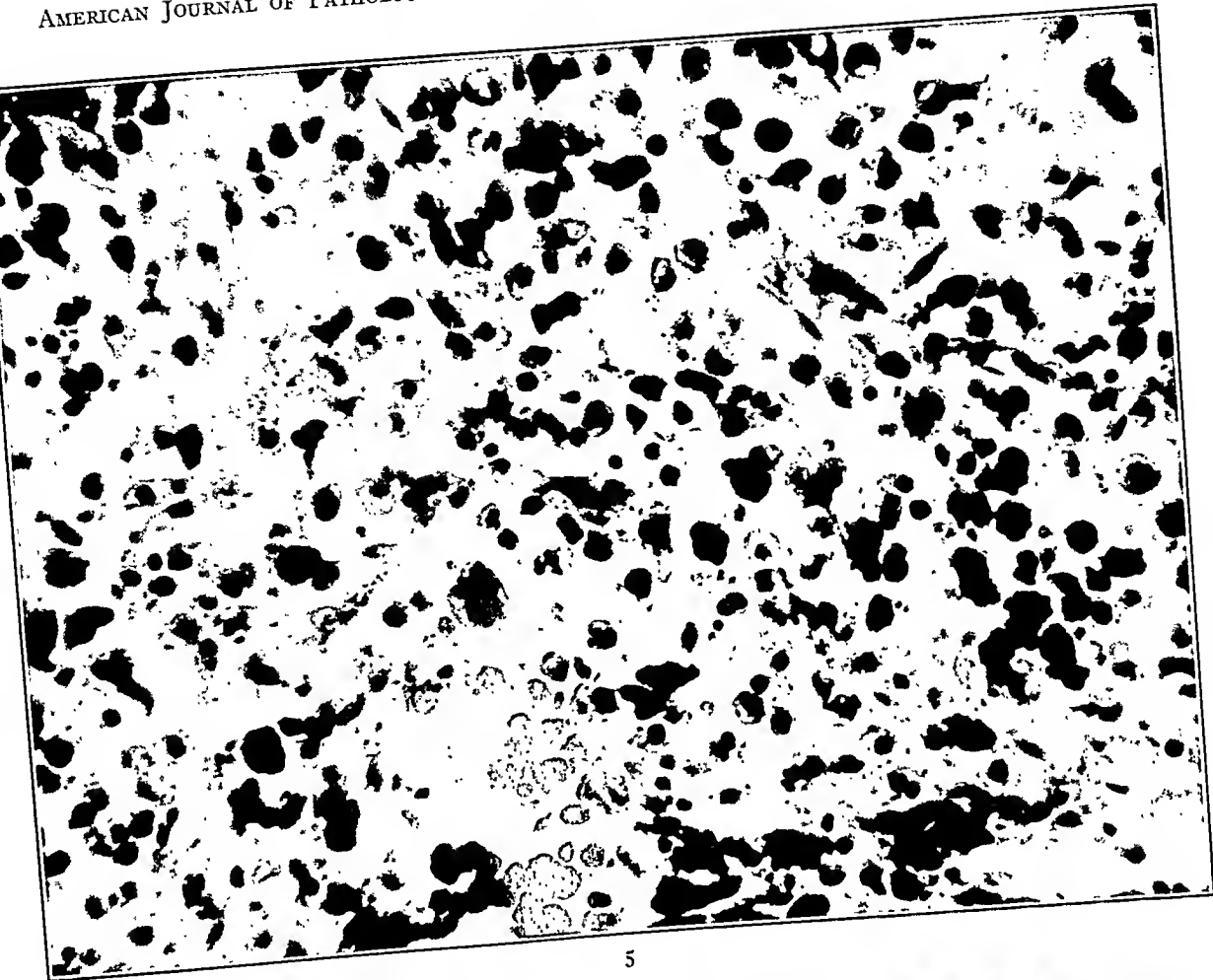


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## PLATE 5

FIG. 5. Case 2. Moderately low power photomicrograph giving a general idea of the varied cell morphology of the tumor. The lightly stained round and oval cells are engorged with fat and cholesterol, the large blackish masses are accumulations of yellowish brown pigment. Scattered spindle cells may be seen here and there. The field does not include any of the giant cells mentioned in the microscopic study.

FIG. 6. Case 2. High power photomicrograph from a portion of the field illustrated in Fig. 5. The majority of the cells have a distinctly vacuolated cytoplasm, all others are heavily impregnated with hemosiderin.



Hunter

Benign Polymorphocellular Tumors of Knee Region



## THE CIRCULATORY PATTERN IN THE ISLANDS OF LANGERHANS \*

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The first complete description of the arrangement of the blood vessels of the islands of Langerhans was made by Kühne and Lea<sup>1</sup> in 1882 in the rabbit. By means of injections these investigators found a network of capillaries in the islands which resembled a glomerulus. They also described multiple afferent arterioles, efferent venules and anastomoses with the adjacent interacinar capillaries. They used only single injections and studied the anatomical arrangement by means of sections. Since the work of Kühne and Lea, numerous investigators have studied the circulatory pattern of the islands in various species of animals with contradictory results. Some maintained that the circulation was entirely venous, others that it was entirely arterial, and some that it was only capillary in nature. A complete bibliography of the work done in this field up to 1906 will be found at the end of Dewitt's comprehensive article.<sup>2</sup> Since that time few investigations concerning the anatomy of the circulation have appeared.

In another communication, observations concerning the circulation of the islands of Langerhans in the pancreas of a living white mouse were reported by one of us (B. N. B.).<sup>3</sup> Capillaries, capillary anastomoses, efferent venules, and in some instances afferent arterioles were described. The following anatomical study was undertaken, first to confirm the observations which were made *in vivo*, and second to determine the relationship between the vascular pattern and the preservation of the islands in circulatory disturbances of the pancreas.

### TECHNIQUE

White mice weighing approximately 10 to 20 gm. were used. Under chloroform anesthesia the thoracic and abdominal viscera were exposed through a midline incision extending from the symphysis

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pubis to the manubrium and two lateral incisions extending from the subcostal angle to the axillae. The four flaps formed by these incisions were retracted and held in place by clamps. The body temperature of the animal was kept constant by means of a warm stage that supported a petri dish in which the animal was placed. After cutting away the tip of the heart a glass cannula attached to rubber tubing was inserted into the left ventricle and tied in place. Then a small opening was made in the right auricle to allow the blood to escape during the perfusion.

The injection of the dyes was then started by means of a syringe. Carmine-gelatin, Berlin blue-gelatin and commercial India ink were used. The consistency of the gelatin masses was made to approximate the viscosity of the blood. After trying different combinations it was found that carmine-gelatin followed by India ink was the most satisfactory for double injection purposes. During the perfusion great care was taken to prevent the entrance of air into the system, especially when the change from the red mass to the black mass was made. This was facilitated by an adapter. The interval between the insertion of the cannula and the beginning of the perfusion was made as short as possible to avoid intravascular clotting. The masses flowed easily with little more than gravitational pressure. In order to obtain the correct distribution of the dyes, it was important to observe the vessels of the viscera closely with magnifying spectacles. The ink injection was discontinued when the smallest visible arterial branches of the mesentery and intestines were black and the corresponding veins were still red. It was essential to keep the masses and apparatus warm during the entire procedure. The viscera were kept from drying by irrigating them with warm normal salt solution.

At the end of the perfusion the whole animal was placed in cold 10 per cent formalin. Later the pancreas was dissected out, dehydrated, cleared in methyl salicylate, and observed *in toto*. Selected pieces containing the islands were teased out, or blocks of pancreas were cut with a safety razor blade. These were studied in a small glass dish containing the clearing reagent. Later, the specimens were mounted in balsam or embedded in paraffin and sectioned. The best specimens were the teased preparations. Thick frozen sections were also made.

## THE CIRCULATION OF THE ISLANDS OF LANGERHANS

For descriptive purposes it is convenient to divide the islands roughly into three groups according to their sizes (Fig. 1). Group I includes islands more than 0.3 mm. in diameter. Group II, the most numerous, includes those between 0.3 mm. and 0.15 mm. Group III includes the smallest recognizable aggregations of islet cells. In Groups I and II the islands are supplied by one or more arterioles which enter a network of capillaries. The latter anastomose freely with the adjacent interacinar capillaries and empty into one or more venules. In Group III, afferent and efferent vessels are not present.

*The Afferent Arterioles:* The afferent arterioles are short, varying from 0.06 mm. to 0.18 mm. in length. In diameter they are slightly larger than the capillaries. They arise in most instances directly from the intralobular arteries, and supply the islands in the central zone of the primary lobules. The islands situated near the periphery of the lobules and in the interlobular connective tissue receive short branches from the interlobular arteries. The number of afferent arterioles varies with the size of the islands. In Group I, two or more arterioles are usually present. In Group II, one and less often two afferent vessels are found. In examining a large series of islands, the smallest one supplied by an arteriole measured 0.15 mm. in diameter.

*Capillary Network:* As the arterioles enter the islands they divide into numerous capillaries which form an interanastomosing plexus. The capillary loops are tortuous, making sinuous curves, spirals and circulets. At frequent intervals, short twigs are given off at various angles forming intercommunications between the loops. This arrangement is characteristic for the islands in Groups I and II. In Group III the capillaries consist of two, three or more tortuous loops which are continuous with the interacinar capillaries.

*Anastomoses:* Rich anastomoses are found to exist between the capillaries of the islands and those in the interacinar rete. It is easy to distinguish the two types of vessels because the former are shaped in the form of loops, whereas the latter have a pattern which conforms to the outlines of the acini.

*Venules:* More than one efferent venule is usually present, depending upon the size of the island. The venules resemble the arterioles in that they are short and empty directly into the intra-



lobular veins; sometimes they join the interlobular and larger pancreatic veins. Instead of leaving the island directly, the venules usually follow the surface of the island for a short distance, receiving numerous tributaries from the insular capillaries along their course. In addition, the efferent venules receive tributaries from the adjacent interacinar capillaries.

## DISCUSSION

The preceding anatomical studies of the circulatory pattern in the islands of Langerhans confirm the observations which were made *in vivo*.<sup>3</sup> The arrangement consists of a network of capillaries which are supplied with blood through afferent arterioles, empty into efferent venules, and anastomose freely with interacinar capillaries.

An analysis of the vascular system of the islands shows that the arrangement of the vessels favors the circulation of a large volume of blood with maximum efficiency. Since the arterial pressure diminishes directly with the length of the vessel, it is higher at the end of a short arteriole than at the end of a longer one, assuming the diameters to be equal. Therefore the short direct arterioles to the islands afford a higher pressure for the column of blood entering the capillary network than the long arterioles supplying the capillaries of the acinar tissue situated at the periphery of the lobules (see Fig. 2). When the capillary bed of the islands is large, more than one arteriole is present. On the venous side, the multiple short efferent venules of the islands insure a low venous pressure with minimal resistance to the egress of blood. Regardless of the position of the islands in the pancreatic lobules, the dynamics of the circulation remain unaltered. These pressure relationships explain why the islands remain intact in advanced chronic passive congestion, whereas the acini in the peripheral parts of the lobules undergo extensive atrophy.<sup>4</sup> On the one hand, the pressure in the short arterioles to the islands is sufficient to overcome the increased resistance in the venous system. On the other hand, the pressure in the arterioles to the outlying acini is insufficient to overcome the venous resistance which is augmented by stasis in the long efferent venules. As a result, the nutrition of the islet cells remains undisturbed while degeneration of the acini occurs. The few acini which remain intact are found in the immediate vicinity of the islands where the circulation is best.

When thrombosis, embolism, sclerosis or spasm of the afferent arterioles occur, the capillary anastomoses can form a collateral circulation which maintains an adequate blood supply to the islands. This mechanism probably accounts for the few changes which are observed in the islands in the presence of arteriolar lesions.

Examination of the circulatory patterns of the islands of Langerhans in the pancreas of the guinea pig, white rat, monkey and man revealed that they were similar to those of the white mouse.

### SUMMARY

1. An anatomical study of the circulatory pattern in the islands of Langerhans was made and the arrangement corresponded with observations made *in vivo*.

2. The importance of the anatomical arrangement of the blood supply in preserving the islands in the presence of circulatory disturbances in the pancreas is discussed.

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## DESCRIPTION OF PLATE

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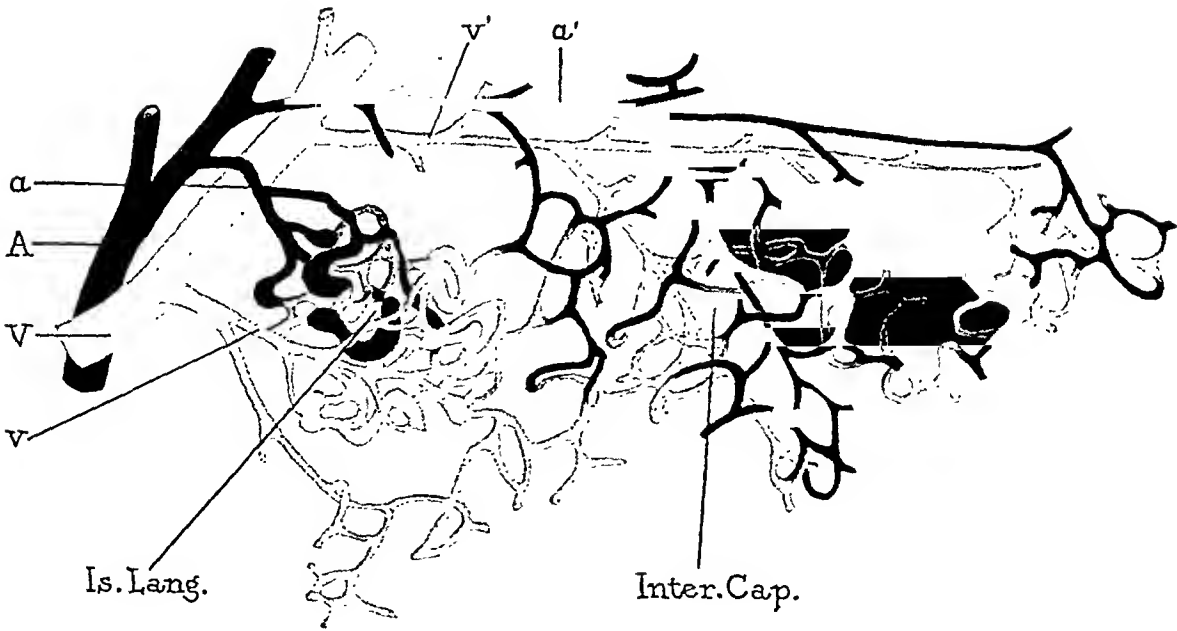
### PLATE 6

FIG. 1. Typical arrangement of capillaries in island of Langerhans. Afferent arterioles (black). Efferent venules (gray). Anastomoses with interacinar capillaries. Camera lucida drawing.  $\times 225$ .

FIG. 2. Schematic drawing of the blood supply of part of a primary lobule of the pancreas showing the circulatory pattern of an island of Langerhans and its relationship to the acinar vessels. (*A*) intralobular artery, (*V*) intralobular vein, (*a*) afferent arteriole, (*v*) efferent venule, (*Is. Lang.*) capillary network in island of Langerhans, (*a'*) small artery supplying acinar tissue, (*v'*) small vein accompanying (*a'*), (*Intcr. Cap.*) interacinar capillary network.



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## INTERACINAR EPITHELIUM OF THE THYROID GLAND \*

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The literature concerning the normal and pathological histology of the thyroid gland is extensive and often controversial. In 1863 Virchow<sup>1</sup> wrote that "Die Follikel, welche die letzten Abschnitte bilden, werden gewöhnlich als runde Blasen beschrieben und abgebildet, indess finde ich, dass die scheinbaren Blasen vielfach unter einander zusammenhängen und verästelte, blasige Auswüchse oder Fortsätze besitzen, welche jedoch selten in einer Ebene liegen und daher je nach der Richtung des Schnittes bald isolierte, bald als verbundene, runde, ovale oder längliche Gebilde von sehr verschiedener Grösse erscheinen." In 1880 Wölfler<sup>2</sup> propounded his hypothesis of undifferentiated rests of fetal epithelium to explain the interacinar epithelium so simply accounted for by Virchow as extra-acinar buds. Subsequent studies of the significance of interacinar epithelium have naturally included the configuration of acini and the mechanism of hypertrophy, and many have differed fundamentally in their conclusions.

It is not probable that Wölfler's hypothesis can ever be entirely disproved. Among others, Wegelin<sup>3</sup> and Marine<sup>4</sup> have treated the theory conservatively and have not denied its plausibility. In reference to interfollicular epithelium Marine has recently said, "These cell rests may be considered as the excess of undifferentiated and vegetative thyroid tissue developed during the period of fetal differentiation and normally destined to undergo gradual absorption, but potentially capable of growth and varying degrees of differentiation whenever a sufficient stimulus for increased thyroid activity is applied."

The problem cannot be solved in examinations confined, as in ordinary sections, to one plane. A three-dimensional study is required to establish positively the continuity or discontinuity of interfollicular epithelium with follicles.

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This mode of study was first employed by Streiff<sup>5</sup> with the wax plate reconstruction method of Born. Streiff reconstructed a part of a single block of tissue from a normal adult thyroid gland. From an examination of this limited material he corroborated a part of Virchow's observation, as indicated by his statement that "*Manche Bläschen weisen sekundäre Ausbuchtungen auf, oder zwei gleich grosse Blasen stehen in offener Verbindung.*" Without a definite statement as to how he made his tracings, the drawings appended to his article indicate that he traced both internal and external surfaces of acini.

Norris,<sup>6</sup> using the wax plate reconstruction method in a study of fetal thyroids, stated that "Secondary follicles are formed from pre-existing follicles apparently by three methods: by solid buds; by hollow buds; and by constriction of the parent follicle." He observed further that although this extra-acinar budding was most active before the fetus had reached 163 mm. in length, it continued throughout fetal life. His reconstructions were also made of both internal and external contours of acini but did not include adult human material.

Wilson<sup>7</sup> studied the thyroid follicle from normal and pathological glands and because he reconstructed internal rather than external contours of follicles, his observations on interacinar epithelium and extra-acinar budding are open to criticism.

Recently Rienhoff<sup>8</sup> has supplemented the wax plate reconstruction method by maceration and microdissection to determine the external configuration of acini. The reconstructions were employed to study the internal form, and the dissected specimens the external forms of follicles. Rienhoff differs from the previous observers in that "There was no evidence of budding, fusion, constriction or division of the follicle when seen and examined in its entirety. . . . It seems evident then that all of these investigations in the past have been concerned chiefly with the individual follicle, and that in them the internal form of the vesicle has been considered the external form or true shape. As a result of this mistake, conclusions as to the continued formation of new follicles in the adult, based on irregularities in the form or shape of the follicles reported in the past are incorrect because the irregularities occur only inside the follicle." He concluded that there were no cell rests either fetal or adult between the follicles and that Wölfler's misconception was based on "tan-

gential sections through the dome of an underlying follicle giving the impression of a group of disconnected epithelial cells."

These investigators (Streiff, Norris, Wilson, Reinhoff) have agreed that there is but little to support Wölfler's hypothesis, but have disagreed fundamentally in regard to the occurrence of external budding of acinar epithelium and the formation of new acini.

### HISTOLOGICAL STUDIES OF THE THYROID GLAND

Blocks of normal thyroid glands, colloid goiters, glands exhibiting active as well as involuting hypertrophy and hyperplasia, nodular (adenomatous) goiters and adenomas, were selected. Many of these blocks were cut in uninterrupted series of sections, some of the series being stained by hematoxylin and eosin and others by the Maresch modification of the Bielschowsky method. It was found necessary to employ the latter method to demonstrate the periacinar reticulum, particularly in hyperplastic glands where the interfollicular stroma had almost disappeared. The serial sections were cut 8 microns thick. To recognize the same structure in succeeding sections of a series, a lobule was chosen as a marker. A lobule could be recognized under low magnification and the change in its contour was sufficiently gradual so that no difficulty was encountered in following it through a series. In blocks of colloid and nodular goiter and in colloid involution of pathological hyperplasia, it was possible to reconstruct from every third or even fourth section, since the changes in acinar contour were more gradual. The drawings were traced from internal and external surfaces of acini from projected images representing a magnification of about 500 diameters.

Wax models were constructed from some of these series. Plates of a thickness corresponding to the magnification were poured and the tracings were transferred to the plates which were then cut. The plates were superimposed to reconstruct the block of thyroid tissue which had been sectioned.

Acinar differentiation was recognized in the thyroid of a 14 cm. fetus, the age of which was estimated to be four months, and which was the youngest fetus included in this study. The thyroid parenchyma was disposed in the form of small, round or irregularly outlined collections of epithelium enclosed by reticulum. Some of these epithelial islands were not solid and contained pale, acidophilic ma-



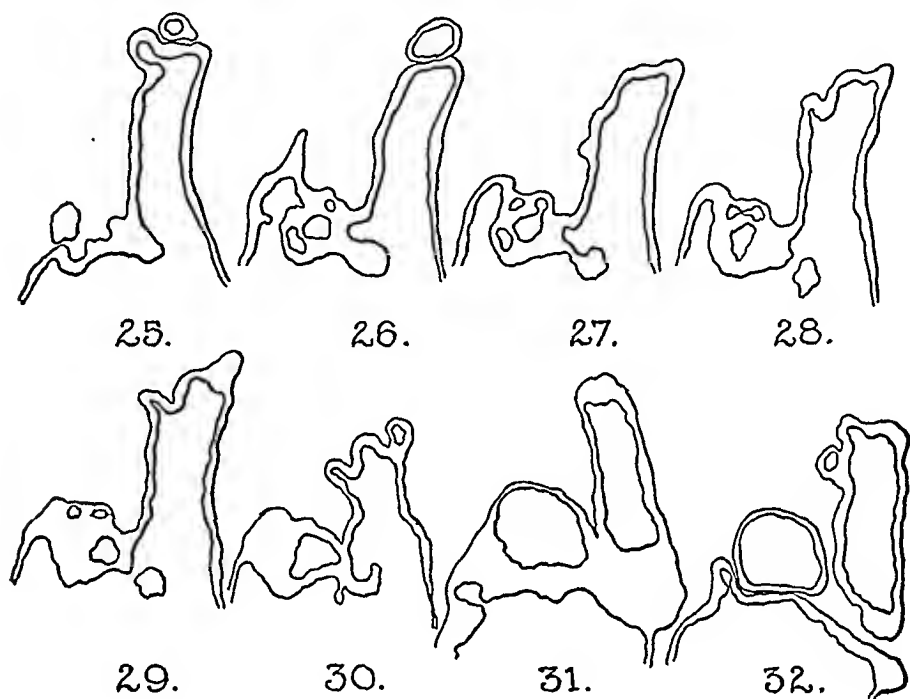
terial. Nuclear polarity did not indicate complete acinar differentiation. Active proliferation was manifested in these cellular islands from many of which buds projected.

In the thyroid of a fetus 20 cm. in length and estimated to be between five and six months of age, acinar differentiation was more advanced. Many of the follicles were lined by cuboidal epithelium exhibiting basal nuclear polarity, were enclosed by periacinar reticulum and contained secretion. Great irregularity in acinar outline was manifest, acini being tortuous, branched structures with solid and hollow extra-acinar buds of epithelium. Such differentiation was not complete, however, and many solid islands of epithelium were encountered. It was apparent that during fetal life new acini were formed from already differentiated acini as well as from undifferentiated epithelium and that the amount of undifferentiated epithelium decreased with the advancing age of the fetus.

A number of normal thyroid glands from children, whose ages ranged from two months to sixteen years, were examined. Some were studied from serial and others from random sections. The islands of undifferentiated epithelium which constituted so prominent a part of the thyroid of the four-month fetus were less conspicuous with advancing age. New acini were formed by intra- and extra-follicular proliferation of epithelium which first formed solid cell masses and later daughter follicles. Solid nests of epithelial cells could be more readily accounted for as the result of extra-acinar hyperplasia of the epithelium lining the acini than as rests of undifferentiated fetal epithelium. These observations were generally in agreement with those of Norris although our studies did not indicate that constriction of follicles occurred. It seemed more likely that the phenomenon described by him as constriction was intra-acinar proliferation of epithelium with formation of secondary follicles within the primary.

In the following consideration of the adult thyroid gland, it may be said that the normal thyroid gland is a labile, everchanging tissue that differs from the pathological gland in degree rather than in kind of change. The definition of a normal gland is necessarily relative and we may say that a gland is normal that conforms in size, shape and structure to the generally accepted average, that generally accepted average to be established from observations in regions not the seat of endemic goiter.

Routine sections of normal (25 to 35 gm.) thyroid glands obtained at autopsy showed that there were scattered foci of actively proliferating glandular epithelium. In a thyroid gland which weighed 30 gm., from a 67 year-old negro who died of syphilitic aortitis, hyperplastic foci were present in what was generally a normal gland. There were areas in which new acini were being formed by intra- and extra-acinar proliferation. Some of these newly formed follicles



TEXT-FIG. 1

Eight tracings of a series from slides which include an area of focal hyperplasia in a normal thyroid. The three follicles seen in section 32 may be traced back to section 30 where their connection with one another is shown. Epithelial proliferation into and out of acini is present.  $\times 125$ .

had acquired their own periacinar reticulum while others had not and were recognized only by the arrangement of their cells.

A study of serial sections through such an area of focal hyperplasia illustrated clearly the manner in which new follicles were formed and also indicated the origin of what in a random section would have appeared to have been undifferentiated interacinar epithelium. Text-Fig. 1 is a part of a series of tracings from a series of sections of a block including such a focus of hyperplasia. New acini were being formed by solid and hollow buds, some of which projected into the

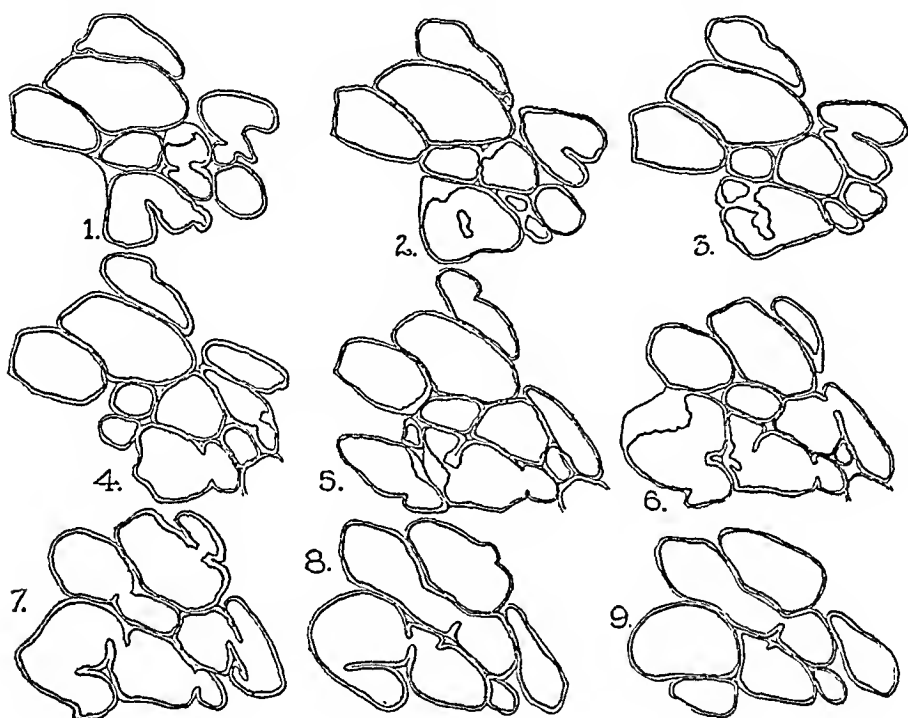
primary acinus while others extended out from it. In section 32 of Text-Fig. 1 there were apparently three distinct follicles which, when traced back to section 30, were seen to be outgrowths of the primary follicle.

The wax model reconstructed from this block of tissue is reproduced in Fig. 1 and the marked irregularity in the external contour of the acinus is seen. Only a segment of the wall of the primary follicle (*a*) is shown, and the follicles marked (*b*) and (*c*) were tubular buds of (*a*). From (*c*) there were a number of small nodular projections of epithelium, some of which were solid and some hollow. The completely detached, solid mass of epithelium (*d*) was supported on a wire strut and may represent undifferentiated interacinar epithelium. It was not possible to prove that such a structure was not a latent rest of fetal epithelium, but it was more easily accounted for as a solid extra-acinar bud which had become detached. Such a cell mass appeared to be a completely undifferentiated and latent structure when stained by hematoxylin and eosin, but with the Bielschowsky method it was seen to have an enclosing reticulum. Often such interacinar epithelium was found to lie within the reticulum enclosing the parent follicle as shown in Fig. 2. It seemed probable that these masses of interacinar epithelium differentiate into follicles which conform to the general structure of the gland.

It was true that most of the solid masses of epithelium encountered in the random section were the result of tangential section through the wall of an acinus and that serial section destroyed the illusion of solidity. Contrary to the observation of Rienhoff, however, there were solid collections of completely detached interacinar epithelial cells.

In agreement with the statement made by Virchow almost ninety years ago, there was intercommunication of follicles to build a labyrinthine system. If it were possible to aspirate the contents of follicle (*a*) in Fig. 1, the colloid would have been withdrawn from follicles (*b*) and (*c*) and others not included by the reconstruction. This does not imply that the entire thyroid is a labyrinthine glandular structure or even an entire lobule. Such intercommunication of acini occurred in areas of hyperplasia. Most of the follicles of the normal thyroid were discrete structures and it was thought that the new acini formed by extra- and intra-acinar proliferation eventually lose their connection with the mother follicle and become discrete.

Intercommunication of acini as a result of coalescence was seen in involuting glands, particularly as a result of iodine therapy. Text-Fig. 2 is a series of tracings of every fourth section from a gland



TEXT-FIG. 2

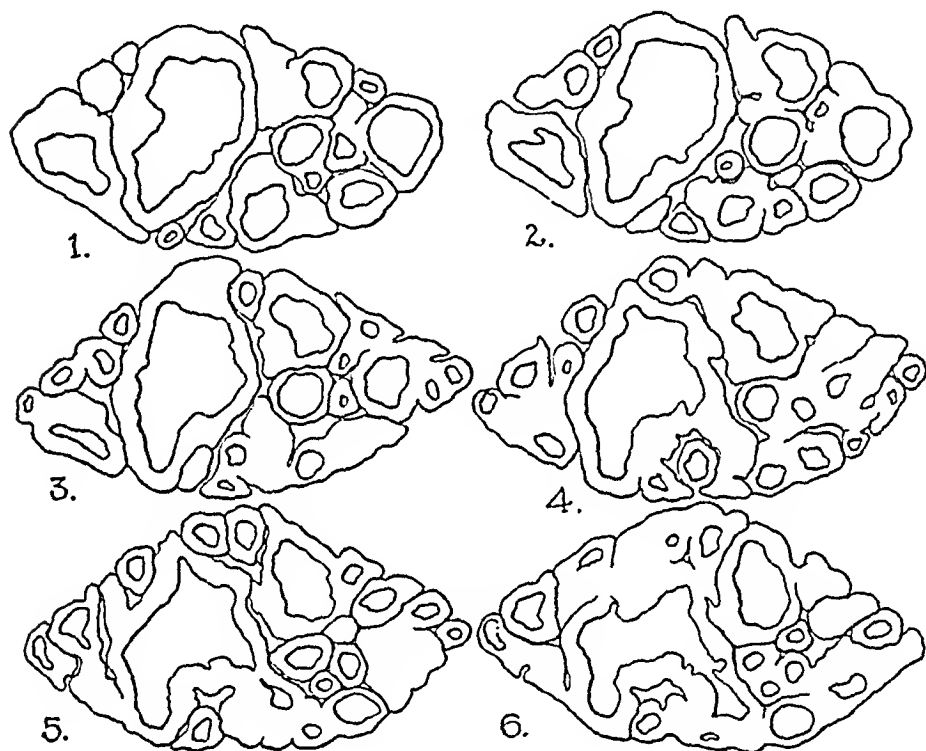
Nine tracings of every fourth section from a series of a block of rapidly involuted thyroid. Confluence of follicles through destruction of acinar walls leaving spurs.  $\times 125$ .

that had involuted rapidly with rest in bed and iodine administration. Coalescence of follicles was prominent and short spurs marked the site of disrupted acinar walls.

The changes seen in pathological hypertrophy and hyperplasia of the thyroid gland differed from the normal in degree rather than in kind of change. Six tracings of a series through such a gland indicated the complexity of the acinar structure (Text-Fig. 3). Many solid masses of undifferentiated epithelium were encountered, some of which were completely isolated structures while others were readily demonstrated to be epithelial outgrowths from acini. Certainly the structure did not conform to Rienhoff's observation that "It is to be noted that except for minor irregularities in contour, the external surfaces of the follicles of the exophthalmic gland are rela-

tively smooth and as observed in the normal follicles, there are no pseudopodial outpouchings of the epithelial sac, no budding off of new follicles, no constriction or cleavage of the primary into secondary follicles."

That pseudopodial outpouching of the epithelium, budding off of new follicles, and formation of secondary follicles by extra- and intra-acinar proliferation does occur, is shown in Text-Fig. 3 and



TEXT-FIG. 3

Six tracings of a series of slides of a thyroid stained by Bielschowsky-Maresch method exhibiting pathological hypertrophy and hyperplasia. Complexity of acinar structure with intra- and extra-acinar proliferation to form secondary follicles. Pseudopodial buds project from acini.  $\times 125$ .

Fig. 3, the latter of which is a photograph of a wax model reconstructed from hyperplastic thyroid gland. This is indicated by Fig. 4 which is a section of hyperplastic thyroid gland stained by the Bielschowsky-Maresch method.

Precisely the same sort of growth activity was seen in the nodules of the nodular or adenomatous goiter and in the encapsulated, distinctly neoplastic, so-called fetal adenomas. Because of the serous

degeneration so often seen in the stroma of adenomas, the extra-follicular budding was readily demonstrated by hematoxylin and eosin stains. Such extra-follicular proliferation has been recently described by Boyd.<sup>9</sup>

### SUMMARY

1. This study indicates that in normal thyroids of fetuses, children and adults, in pathological glands the seat of hypertrophy and hyperplasia, in nodular goiter, and in adenomas of the thyroid, the mechanics of hyperplasia are essentially similar.

2. New follicles are formed by intra- and extra-follicular proliferation of epithelium and the secondary follicles may lose their parent connection and become isolated units.

3. In the scattered foci of hyperplasia in normal thyroid glands, as well as in glands that are the seat of pathological hypertrophy and hyperplasia, labyrinthine intercommunication of acini exists and is probably limited to the connection of primary with secondary acini.

4. Solid masses of undifferentiated interfollicular epithelium are found in both normal and pathological glands, but such masses are readily accounted for as detached extra-follicular buds.

5. Evidence contrary to certain observations by Rienhoff concerning the mechanics of hyperplasia in the thyroid gland is presented.

I wish to express my appreciation to Dr. H. T. Karsner for his advice and helpful criticism.

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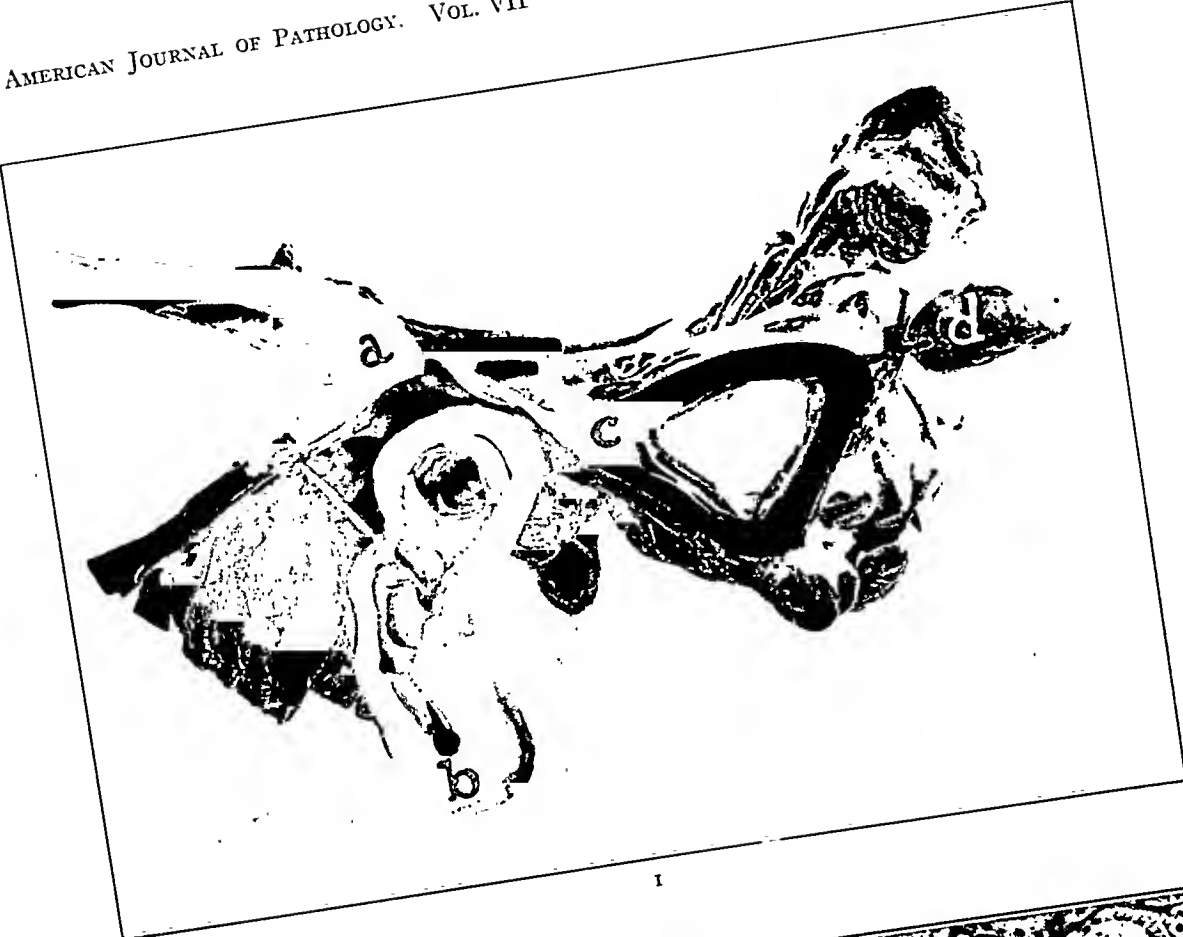
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## DESCRIPTION OF PLATES

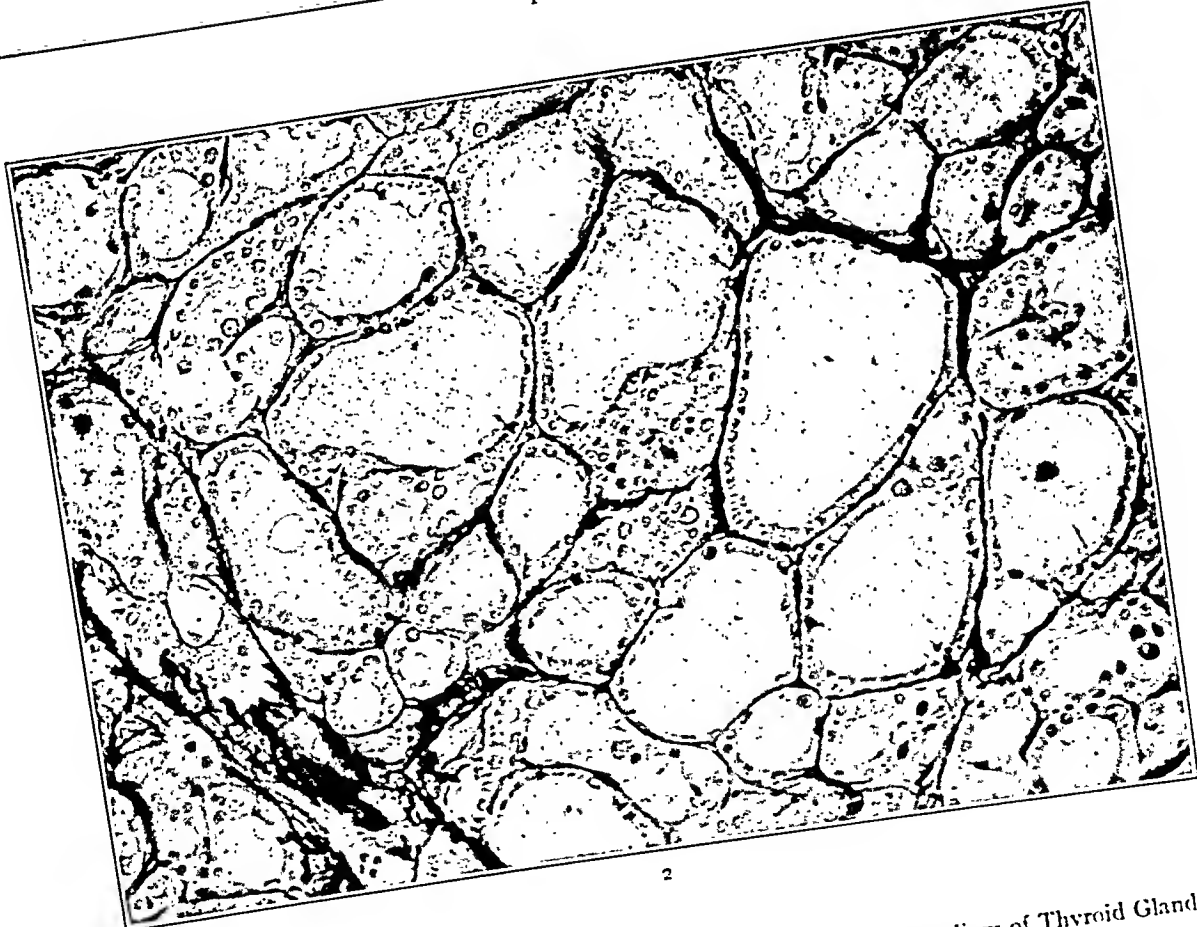
### PLATE 7

FIG. 1. Wax model constructed from tracings of serial sections from block of normal thyroid including an area of focal hyperplasia. (a) Primary follicle. (b) and (c) Secondary follicles which communicate with (a). (d) Solid nest of undifferentiated interacinar epithelium. Model represents an enlargement of 500 diameters. Reproduction is one-half actual size of model.

FIG. 2. Hyperplastic adult thyroid gland. Solid mass of epithelium included within periacinar reticulum. Bielschowsky-Maresch stain.  $\times 300$ .



1



2

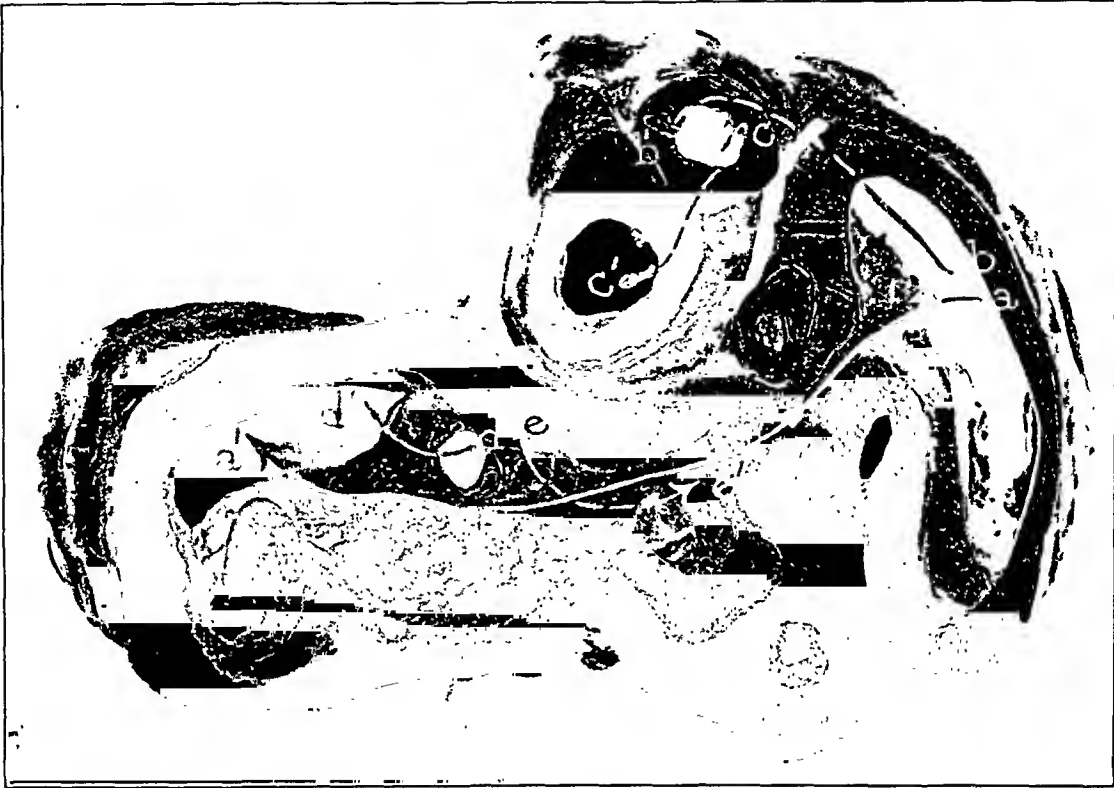
Interacinar Epithelium of Thyroid Gland



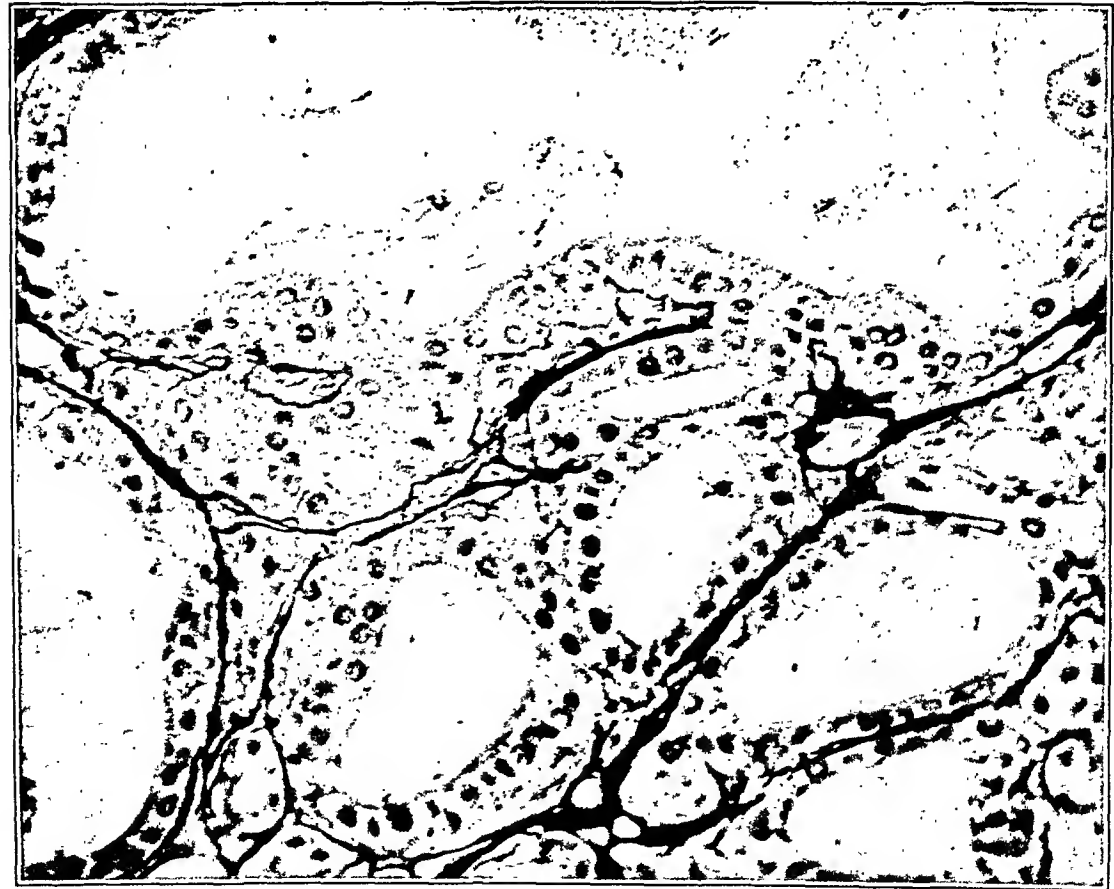
## PLATE 8

FIG. 3. Wax model constructed from thyroid exhibiting pathological hypertrophy and hyperplasia.  $a - a'$ ,  $b - b'$ ,  $c - c'$ ,  $d - d'$  and  $e - e'$  mark interacinar communications. Model represents an enlargement of 500 times. Reproduction is one-half actual size of model.

FIG. 4. Formation of secondary follicles by extrafollicular budding. Some of the daughter follicles have not yet acquired an enclosing reticulum. Small solid bud shows beginning acinar differentiation. Bielschowsky-Maresch stain.  $\times 450$ .



3



4



# PRACTICAL SUGGESTIONS FOR SILVER IMPREGNATION OF CONNECTIVE TISSUE \*

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Impregnation of connective tissue with ammoniacal silver is the most delicate and complex of all histological techniques, but if we take advantage of it, this very complexity renders the method manifold in its applications and fertile in its results. Having acquired a certain skill from long practice, we shall summarize here the lessons of experience.

There are few methods less suited to rigid rules than silver impregnations. To obtain from them all that they are capable of giving, the worker should not rely on strict formulas of time and proportion. The automatic application of no matter what silver technique cannot lead to constant results. To the mishaps thus experienced we may attribute the multiplicity of methods proposed, no one of which is universal or infallible. The picture changes, however, if we bear in mind that the object is not to follow a given silver technique with blind confidence, but to color the sections. This is especially true if we look on each tissue to be studied as a special problem requiring individual treatment with all that this word implies of observation, understanding, interpretation of the facts and adaptation to the actual circumstances.

We shall have attained our object if we can convince the reader that, far from being guided by strict and invariable rules, at each step the worker should survey and know how to interpret the successive results of each manipulation. This is advisable in order to modify, in a direction which it is necessary to discover, the next steps in the technique. No method requires more liberty of procedure, flexibility and initiative on the part of the worker.

## FIXATION

In Bielschowsky's original method and in the early techniques inspired by it, the tissue was fixed exclusively in formol. As a matter of fact the methods of impregnation with ammoniacal silver are

\* Received for publication October 2, 1930.

sufficiently flexible to succeed, after some modifications, with tissue that has been fixed in many ways. One might think that formol fixation favors the impregnation because there is no intervention of a metallic salt, but such salts as sublimate, chromates and uranium nitrate \* do not hinder the impregnation in any way.

Fixation with bichromate-formol-uranium (Tupa's fluid)† has enabled us to impregnate the reticulin of the interscapular brown fat (hibernating gland) of the white rat. The impregnation was particularly successful in the superficial zones where this excellent but slightly penetrating fixative acted correctly. This fluid has given us the best fixation, the most perfect preservation and the purest impregnation of the finest fibrils of the subcutaneous connective tissue.

With Zenker's fluid, Laidlaw obtained his well known brilliant results. We have used Zenker's as well as Helly's fluid and have found them very satisfactory.

Bouin's fluid, even when it has acted for an incredibly long time, permits the impregnation of the reticulin with no preparation other than the removal of the picric acid by thorough washing in running water. This is shown in Fig. 3, elephant's liver that had been preserved in Bouin from 1923 to 1930, an anatomical specimen which we owe to the kindness of Professor Anthony of the Paris Museum of Natural History.

With the piece of elephant's liver, Professor Anthony gave us tissue from another anatomical specimen, a hippopotamus liver that had been preserved in formol since 1917, that is to say, for thirteen years. The disorganization of his service during the war had not permitted as prompt an autopsy as would have been desired. At the time of fixation the liver was already in such advanced putrefaction that bubbles of gas have left their mark in the tissue. Despite such unfavorable conditions, the reticulin impregnates well with no preparation other than treatment of the sections for a week with Gram's solution. The preparations thus obtained were finer than those from the other half of the piece which had been subjected to rejuvenation by ammonia water followed by refixation in Zenker's fluid, as advised by Davidoff.

\* Already used by Cajal, but with silver nitrate, not with ammoniacal silver.

† Potassium bichromate 3 per cent.....	80 cc.
Formol.....	20 cc.
Uranium nitrate.....	1 gm.

Passage of the tissue through 4 per cent sodium hydrate, which destroys the protoplasm leaving nothing but the framework, does not in any way hinder the impregnation of the reticulin after fixation in formol or in Helly's fluid, results contrary to those obtained by Foot who was convinced that there is an argyrophil substance dissolved and removed by the soda.

Finally, the reticulin impregnates just as well in tissue that has not been fixed at all. Frozen sections of fresh tissue received in Locke's solution, then washed in distilled water, give very pure and perfect impregnations (Fig. 1).

Laidlaw has shown the importance of regulating the duration of the fixation to avoid the staining of confusing elements such as the neurokeratin of the nerve fibers. Our fixation times have been the usual ones, 6 to 12 hours in bichromate-formol-uranium, Zenker's or Helly's fluids, 1 week in formol, 2 to 3 days in Bouin.

### CUTTING THE SECTIONS

Sections may be made in the usual manner. After formol fixation we make frozen sections, for formol does not fix the tissue hard enough to prevent damage to the finer fibrils by the heat of paraffin embedding. After the other fixatives, embed in paraffin or celloidin. Paraffin would seem to be the easier, but celloidin, just because it avoids the heat of the paraffin bath, is more sure to preserve the finest fibrils. Besides, celloidin permits thicker sections, exhibiting more of the framework, and allows the examination of layers quite distant from the surface of the section, which is always more or less disarranged. This is a great advantage, on condition of course that the preparations are very transparent. If, after fixation, one-half of Ranvier's edematous tumor be embedded in paraffin and the other half in celloidin, comparison of sections from each half will leave no doubt on this point.

The thickness of the sections should be proportionate to the density of the tissue, 10 to 15 microns for nerves, muscle, spleen and brown fat, 20 microns for liver with its larger cells, 30 to 40 microns for white fat, 50 microns for the edematous tumor.

To prevent floating off in the hot silver, paraffin sections are stuck to the slide with gelatin, made insoluble during drying by formol fumes (Masson's method).

## PREPARATORY TREATMENT OF THE SECTIONS

Frozen sections of formol-fixed tissue are simply washed in three or four changes of distilled water to remove all trace of the fixative. Sections of Bouin-fixed tissue are washed in running water until the picric acid is removed completely, then passed through distilled water. Sections of tissue fixed in Helly's, Zenker's or bichromate-formol-uranium solutions, and sections that have been preserved for a long time in formol, no matter what the cutting method (frozen, paraffin or celloidin), are treated with iodine in the form of Gram's solution. The sojourn in iodine, never less than overnight, may be prolonged with advantage to three or four days or even one week. The iodine is removed by passage through sodium hyposulphite followed by thorough washing in running water.

When dealing with cells or their prolongations with a particular affinity for silver, such as those of the spinal ganglia or the nerves, or even in tissues where many small cells with their black nuclei tend to mask a delicate reticulum, as in the splenic pulp, it is well to follow the iodine and hypo with potassium permanganate and oxalic acid, as used by Foot and Laidlaw. This treatment, which aims at eliminating the chromate fixed on the protoplasm, diminishes the affinity of the cytoplasm and the nuclei for silver and accentuates the desirable contrast between the reticulin and the background. It should be followed by prolonged washing, observing the usual precautions to avoid the formation of calcium oxalate if the running water at hand is calcareous.

## THE SILVER BATH

All the various methods of preparing ammoniacal silver are about alike. The best is that with which one has the most experience. Knowing its resources and its weaknesses, the worker will know best how to adapt it to each preparation.

For a long time we precipitated the silver with sodium hydrate. To 15 cc. of a 10 per cent solution of silver nitrate, add strong solution of sodium hydrate of any convenient concentration until no more precipitate is formed. Wash the precipitate with distilled water 7 or 8 times, shaking well and letting the precipitate settle to the bottom of the tube each time. The excess sodium hydrate and the newly formed sodium nitrate are thus eliminated. The precipitate,

suspended in 30 cc. of distilled water, is dissolved with ammonia, added very slowly and gradually in the course of an hour. To be sure not to add ammonia in excess, we avoid dissolving the last particles of precipitate, removing them by filtration just before use. Add distilled water up to 60 cc.

At present we prefer a silver solution prepared with lithium carbonate. It has seemed to us that ammoniacal silver carbonate is more stable and keeps for a longer time than the ammoniacal silver hydroxide obtained with sodium hydrate. Dissolve 5 gm. of silver nitrate in as little distilled water as possible and add a saturated solution of lithium carbonate in distilled water until there is no more clouding of the supernatant fluid. Wash the precipitate 7 or 8 times before dissolving it with ammonia. Add distilled water up to 50 cc. Dilute to about one-fourth strength just before use.

The dilute solutions are better than the stronger mixtures. They can be left to act for a longer time, permitting better management of the effect. The silver oxide obtained with sodium hydrate has seemed to us less stable and in consequence more rapid and intense in its action than the silver carbonate prepared by Rio-Hortega's method, using either sodium carbonate or lithium carbonate.

### SILVER IMPREGNATION

To reveal particularly delicate structures such as the finer fibrils of the subcutaneous connective tissue which are infinitely more delicate than the reticulin of such organs as the liver and spleen, the impregnation is best made at room temperature. The results are incomparable.

Celloidin sections are always better treated cold. With equal intensity of coloration, when stained in hot silver the celloidin is likely to become brittle, not always without risk to the preservation of the tissue. It is much better to let the silver act slowly, more than an hour perhaps, at room temperature in small uncovered dishes. If the dish is covered the impregnation takes place still more slowly. After a certain time a film of metallic silver is seen to form on the surface because the ammonia which held it in solution has evaporated. As soon as the film appears the sections should be passed on to the reducer. In waiting longer there is little gain in intensity of the impregnation, while there is risk of precipitation on the upper surface of the section with more tenacious insolubility of the celloidin.



Frozen sections and paraffin sections stuck on the slide are treated hot, always in a closed vessel and at a temperature no higher than 50° C for fear of serious damage to the tissue. Covering the vessel prevents too rapid evaporation of the ammonia with consequent precipitation of metallic silver and the formation of a film on the surface of the fluid before impregnation is complete.

It is illusory to give precise times for the treatment of the sections with the silver solution either in the oven or when heated over a Bunsen flame with interposed wire gauze. The time varies not only for each fixation and for each organ but also for each specimen of the same organ removed from different animals of the same species, and all the more with different species. We must depend on the color assumed by the sections, which varies with their thickness and the richness of the tissue in cells and consequently in nuclei. The only satisfactory method is to put several sections from the same block in the same silver bath, to begin reduction tests when the sections have become medium brown, and to regulate the procedure by the result of these trials.

### RINSING

Rinsing the sections after the silver bath and before reduction in formol is the most delicate step in the method and the one in which the end results may be best controlled by appropriate modifications. The ideal end result is an almost exclusive impregnation of the reticulin traced sharply on a background as colorless as possible, in which only the nuclei are visible or, rather, outlined. In rinsing the sections the chief object is to remove the excess of silver solution which, reduced by the formol, would give superficial precipitates that obscure the details. At the same time, the rinsing should leave a little ammonia in the depth of the tissue, a procedure that is very important for perfect reduction. In fact, it is indispensable that the sections should be impregnated with a certain quantity of ammonia when they come in contact with the reducing formol.

The rinsing, then, should be just sufficient to remove the excess of silver without removing this necessary ammonia. If prolonged, there is danger of modifying the character of the reduction. The simplest way to attain the desired result is to rinse the sections in weak ammonia water before reducing in formol. The usual mixture is 5 drops of ammonia to 50 cc. of distilled water. Since the ammonia

decolorizes the impregnation somewhat, as shown by bleaching the sections, it is advisable to overstain a little. The bleaching with ammonia is distinctly selective; the nuclei and especially the protoplasm are decolorized considerably before the reticulin is affected. This selective action of ammonia water should be resorted to whenever there is difficulty in obtaining a strongly colored reticulin on a pale background (Figs. 2 and 4).

The strength of the ammonia water and the time of rinsing should be in proportion to the thickness of the section and the richness of the tissue in cells. Thin sections of loosely-woven tissue should be rinsed quickly, about 5 seconds, in very weak ammonia water, only 2 or 3 drops to 50 cc. Sections of open networks, such as liver or spleen that have been treated before fixation with sodium hydrate to destroy the protoplasm, preserving only the framework of reticulin, should be simply dipped in the ammonia water and removed immediately, passing to the reducer without loss of a second. Often it is better not to rinse them at all. In this event, to prevent the precipitation favored by such brief or omitted rinsing, a weaker solution of formol, 1 per cent, should be used. With thick sections of dense tissue such as liver and spleen fixed in the usual way, the ammonia should be increased to 7 drops and the rinsing may be prolonged to 10 or 15 seconds, until the section is distinctly but slightly lighter in color. With comparatively impermeable tissue, such as the clots of artificial collagen obtained according to the directions given by Nageotte, the rinsing must be prolonged and the formol solution should be rather weak to prevent as much as possible the formation of precipitates in the deeper layers of the sections, the layers where the rinsing has not sufficiently removed the excess of silver. The superficial layers, subjected longer and more directly to the action of the ammonia, will usually be rinsed too much and will appear less deeply colored than the deeper layers, where the best pictures should be sought.

### REDUCTION

Reduction is carried on in a more or less concentrated bath of formol. The concentration of the formol should be regulated by the duration of the rinsing and by its intensity, which depends on the strength of the ammonia water. Moderate rinsing of 10 seconds or so in water containing 5 to 7 drops of ammonia to 50 cc. should be

followed by reduction in 15 to 20 per cent formol. Relatively thick preparations and those of less permeable nature, such as the clots of artificial collagen, are reduced in weaker solutions of formol. The shorter the time of rinsing in ammonia water, the weaker should be the formol solution. Five per cent formol is often advisable. Contrariwise, very permeable and delicate preparations, such as sections of the reticulum of the spleen or liver almost freed from the cellular elements by the action of sodium hydrate before fixation, should be reduced in 1 per cent formol after very quick rinsing or no rinsing at all.

If the formol used is very acid, the ammonia in the wash water should be increased. The object of the usual recommendation to dilute the formol with tap water is to neutralize partially this acidity of the formol. We prefer distilled water for this purpose to prevent the precipitating action of the lime salts being added to that of the formol.

Naturally, the thicker and less permeable the sections, the longer will be the time required for reduction. During the entire time of the reduction, the sections or the slides should be agitated in order to renew the fluid in contact with the tissue and to remove the precipitate which forms around the sections.

### TONING AND FIXING THE SECTIONS

We need not describe the well known technique of toning with gold. The aim of this procedure is merely to give prettier preparations without making them any more instructive. It may even have the serious defect of enfeebling or removing the impregnation of the finer fibrils. With very delicate preparations it is better to omit toning with gold and fixing with hypo and to mount the sections directly from the formol bath.

### MOUNTING THE SECTIONS

Paraffin sections are mounted in the usual manner, a quick rinse to remove the formol, dehydration in 90 per cent alcohol, then absolute alcohol, xylol, balsam.

Frozen sections are washed and drawn up on a slide perfectly free from grease which is slipped under them in the wash water. The section is spread in a suitable position on the slide, held in place by a

needle applied to a point on its upper border (a point in the preparation which is sacrificed) and the slide is drawn gently from the water, avoiding wrinkles. Dehydrate, observing the precautions necessary with sections unattached to the slide.

Celloidin sections should be washed more carefully for the reducing formol seems to make the celloidin more difficult of solution. The section is drawn up on the slide, care being taken to apply to the slide the surface that was uppermost in the reducing bath. This surface often bears non-adhering precipitates deposited there during reduction and for a few minutes thereafter. If this surface is placed undermost, there is a chance that the precipitate will be rubbed off during the dissolving of the celloidin. If this precaution be neglected the precipitates are liberated during the removal of the celloidin but for the most part they fall back on the section, adhere to the network and can no longer be removed.

The celloidin is dehydrated with absolute alcohol and dissolved by alcohol-ether frequently renewed. If this is ineffective, use acetone. The worker should not be surprised by the rapid evaporation of the acetone, which volatilizes much more quickly than alcohol-ether. Before using acetone, the section should be dehydrated thoroughly for the action of acetone on sections taken from water is disastrous. The acetone, which is rarely perfectly anhydrous, is followed by another absolute alcohol, then by an essential oil (oil of cajeput) and finally by xylol, or directly by carbol-xylol followed by a final rinse in pure xylol before mounting in balsam.

#### INFORMATION FURNISHED BY PRELIMINARY TRIALS OF REDUCTION

In silver impregnations, one can never be sure of succeeding in the first trial. The first section is reduced empirically and the results obtained serve as a guide in treating more judiciously but without delay other sections of the same batch that have been impregnated along with it.

The nuclei are the first elements stained by ammoniacal silver. After washing in ammonia water and reducing, coloring of the nuclei alone means that the impregnation has not been carried on long enough. The other sections of this batch should remain longer in the silver bath.

Overstaining of the cytoplasm may indicate insufficient washing of the sections before immersion in the silver bath, which, in this event, is clouded too much and too quickly by the formol carried along by the sections; or it may mean insufficient washing in ammonia water before reduction. Among the overstained cells, the fine reticulin is distinguished with difficulty.

If, after reduction, normal reticulin appears as a row of tiny black granules instead of clean-cut brownish black filaments, the ammonia water has been too strong and its action too prolonged. Reduce both the time and the concentration.

A general pale brown reduction indicates too prolonged rinsing after silver. If the sections are particularly thin and permeable, a trial may be made of omitting the washing; then reduce in weak formol, agitating the sections constantly in the reducing fluid.

Occasionally after the silver it is well to wash the sections for some time in distilled water and then dip them for a second in ammonia water before reduction in moderately strong formol, about 10 per cent. The connective tissue bundles will be impregnated black.

In truth, each piece demands special treatment, to be obtained by varying this or that step of the technique, the preparatory treatment of the sections, choice and concentration of the silver bath, temperature, duration and intensity of the impregnation, rinsing, and concentration of the formol reducer. With each specimen, long experience with silver impregnations is the only guide as to which side of the line, by trials, we must seek the lucky modification that will produce the result desired.

## DESCRIPTION OF PLATE

### PLATE 9

FIG. 1. Calf's liver. No fixation whatever. Frozen section 15 microns thick.  $\times 250$ .

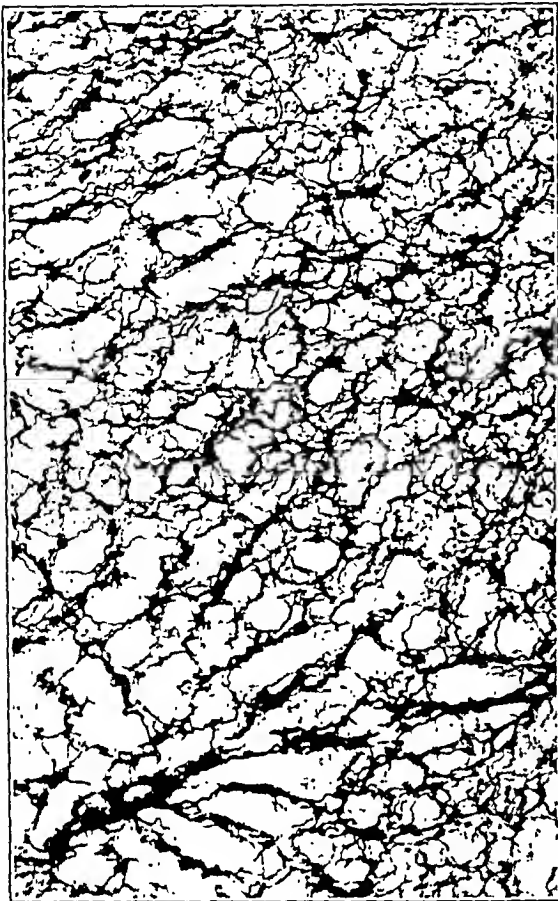
FIG. 2. Horse's spleen. Fixation: Zenker's fluid. Paraffin section 10 microns thick.  $\times 500$ .

FIG. 3. Elephant's liver. Fixation: Bouin's fluid from the year 1923 to 1930. Paraffin section 10 microns thick.  $\times 250$ .

FIG. 4. Dog's liver. Fixation: 15 per cent formol. Frozen section 20 microns thick.  $\times 250$ .

(A) Impregnation in ammoniacal silver carbonate, rinsed in *ammoniacal* distilled water, reduced in 10 per cent formol.

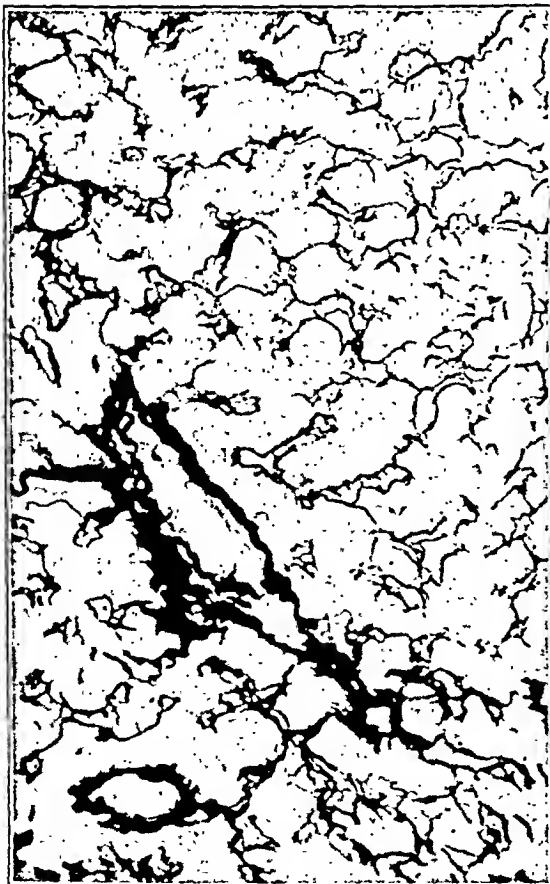
(B) Same impregnation for same time but rinsed in *pure* distilled water, reduced in the same 10 per cent formol.



1

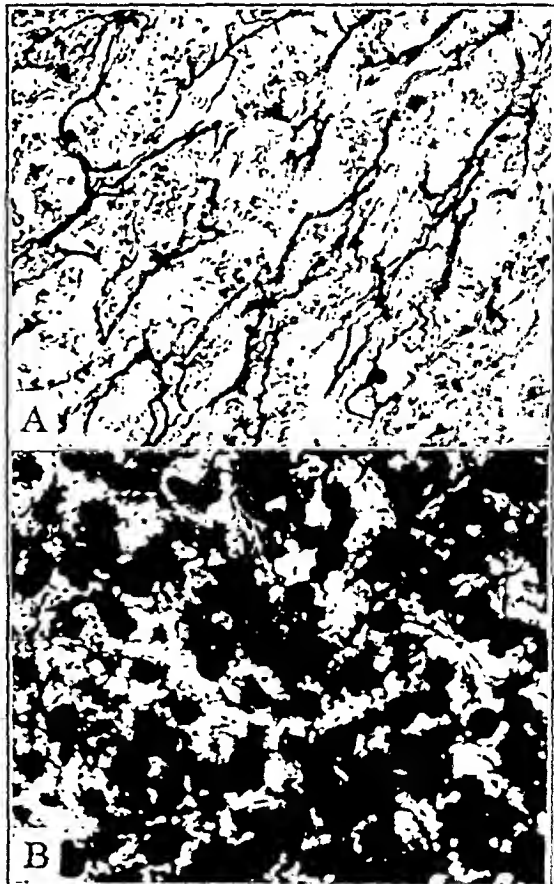


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Guyon



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Silver Impregnation of Connective Tissue



## EXPERIMENTAL GLOMERULONEPHRITIS IN A MONKEY \*

E. T. BELL, M.D., AND B. J. CLAWSON, M.D.

(From the Department of Pathology, University of Minnesota, Minneapolis, Minn.)

This experiment deals with a monkey which was given repeated intravenous injections of streptococci over a period of four years. It was thought that repeated infections over a long period of time might simulate the conditions under which chronic glomerulonephritis develops in man.

A large male rhesus monkey was selected. Before the experiment was begun the urine was examined repeatedly and found free of albumin, erythrocytes and casts.

The organism used was a stock culture of *Streptococcus viridans* which was originally obtained from the blood of a patient with acute rheumatic fever. The bacteria injected were taken from agar slants incubated twenty-four hours at 37°C. Suspensions of one or more agar slants were made in 10 cc. of normal salt solution. The size of the injection is indicated in the table, + indicating one agar slant, ++ two or three slants, and +++ five slants. The dosage varied somewhat with the amount of growth on the agar.

The urine was collected in a metabolism cage. The animal was not catheterized. The amount of albumin is indicated roughly in the table, + indicating a trace, ++++ when the urine was semisolid on boiling, and ++ and +++ intermediate amounts.

The amount of gross blood is indicated roughly in the table, +++ representing a definitely red color, and + a barely visible redness. Small amounts of blood were frequently checked by microscopic examination.

The injections were discontinued occasionally because of the physical condition of the monkey, since we were anxious to keep the animal alive as long as possible. During the latter part of the experiment particularly, the injections were usually followed by severe reactions during which the animal often seemed near death for several hours. When albuminuria decreased or disappeared, heavier and more frequent injections were given. During the last two years of the experiment only occasional small injections were sufficient to keep up a persistent albuminuria.

\* Received for publication October 30, 1930.



Gross blood was present in the urine on 50 out of 119 examinations. It was found much oftener during the latter half of the experiment. The amount of blood was greatest immediately following an injection, after which time it usually decreased gradually.

No edema was present at any time. The blood pressure was not recorded. During the last year of the experiment the animal gradually lost weight and finally became emaciated.

The details of the experiment are given in the following table:

The postmortem examination was made a few hours after death. Definite emaciation was noted. There was no subcutaneous edema, and no fluid was found in the serous cavities. The heart was small, indicating that hypertension had not been present. The lungs were entirely normal. No tuberculosis was present in any part of the body. No disease was found in any organ except the kidneys.

The kidneys were slightly enlarged, weighing together 44 gm. The external surfaces were smooth. On section the cortices were of pale color, not yellowish. Microscopic sections stained with scarlet red showed only occasional fat droplets in the tubules.

Under low magnification a moderate tubular atrophy is found throughout the kidneys. This is indicated by the increased connective tissue between the tubules. The atrophy is diffuse but is more pronounced in some areas than in others. Fig. 1 shows an area with rather conspicuous tubular atrophy.

None of the glomeruli are hyaline, but they all show definite narrowing and occlusion of the glomerular capillaries (Fig. 2). This appearance is present in every glomerulus and therefore cannot be interpreted as a focal lesion. The glomeruli are not enlarged. Very few erythrocytes are seen because of the narrowing and closure of the capillaries. Many of the glomerular lobules have a hyaline appearance.

It is easily seen with the iron-hematoxylin stain (Fig. 2) that there is a widespread obstruction in the glomerular circulation which is sufficient to explain the tubular atrophy. But this stain does not show the details of the glomerular lesion.

Sections stained by McGregor's method (Heidenhain's modification of Mallory's anilin blue) show the nature of the capillary obstruction. In Fig. 3 it is seen that the chief cause of capillary obstruction is a marked increase in the number and size of the capillary

TABLE I

*Experimental Data on Monkey Injected with Streptococcus Viridans*

Injections		Urine examination			Injections		Urine examination		
Date	Size	Date	Albu- min	Gross blood	Date	Size	Date	Albu- min	Gross blood
12/21/25	+	12/23/25	-	-	6/3/27	+	6/6/27	+	-
12/28/25	+	..	..	..	6/11/27	++	6/13/27	+++	-
12/31/25	++	1/2/26	+++	-	6/17/27	++	6/18/27	+++	+
1/4/26	++	1/6/26	-	-	..	..	6/26/27	++	-
1/8/26	++	..	..	..	6/27/27	+	6/28/27	++++	-
1/12/26	+	1/13/26	-	-	..	..	6/30/27	++++	+++
1/15/26	++	1/16/26	-	-	..	..	7/1/27	+++	+++
1/18/26	++	1/26/26	-	-	..	..	7/5/27	++	-
1/28/26	++	1/30/26	-	-	..	..	8/30/27	-	-
2/1/26	++++	2/3/26	-	-	9/9/27	++	9/10/27	++++	-
2/5/26	++++	2/5/26	-	-	..	..	9/12/27	+	-
2/11/26	++++	2/13/26	+	-	9/15/27	++	9/16/27	-	-
2/15/26	++++	2/16/26	-	-	..	..	9/19/27	+++	-
3/15/26	++++	3/16/26	++	-	9/22/27	++	9/22/27	+++	-
3/23/26	++++	3/25/26	++	+	..	..	9/23/27	++++	+++
4/9/26	++++	4/12/26	++++	+++	..	..	9/24/27	++++	+++
..	..	4/15/26	++	-	..	..	9/26/27	+++	+++
4/16/26	++++	4/17/26	++	-	9/28/27	++	9/30/27	++++	+++
4/21/26	++++	4/24/26	++	+	..	..	10/1/27	++++	+++
5/4/26	++++	5/5/26	++++	+++	..	..	10/3/27	++++	+++
..	..	5/11/26	+++	++	..	..	10/5/27	++++	++
5/12/26	++++	5/18/26	++	++	..	..	10/8/27	+++	-
5/22/26	++++	5/24/26	++	++	10/10/27	++	10/11/27	++++	+++
..	..	5/29/26	++	+	..	..	10/12/27	++++	+++
6/5/26	++++	6/7/26	++++	+++	..	..	10/13/27	++++	+++
6/12/26	++++	6/14/26	++	+	..	..	10/15/27	++++	+++
6/17/26	++++	6/21/26	+	-	..	..	10/18/27	++++	+++
6/22/26	++++	6/23/26	++	+	..	..	10/21/27	+++	-
6/24/26	++++	6/26/26	+	-	..	..	10/25/27	++	-
6/29/26	++++	6/30/26	+	-	..	..	10/28/27	++	-
7/2/26	++++	7/3/26	++	-	10/30/27	+	11/1/27	+++	-
7/9/26	++++	7/10/26	+	-	..	..	11/2/27	++++	+++
9/20/26	++++	9/21/26	-	-	..	..	11/4/27	++++	+
..	..	9/24/26	-	-	11/14/27	+	11/14/27	++	-
10/1/26	++++	10/2/26	-	-	..	..	11/15/27	++++	+++
10/8/26	++++	10/9/26	-	-	..	..	11/18/27	++++	+++
10/22/26	++++	10/23/26	++++	+++	..	..	11/23/27	+++	+++
10/28/26	++++	10/30/26	++++	++	12/3/27	++	12/5/27	++++	+++
..	..	11/5/26	+	-	..	..	12/7/27	+++	+++
11/10/26	++++	11/11/26	++	+	..	..	1/3/28	++	-
..	..	11/13/26	++	++	1/9/28	+	1/10/28	++++	++
11/19/26	++++	11/20/26	++	+	..	..	1/12/28	++++	+++
..	..	11/26/26	-	-	1/23/28	++	1/25/28	++++	+++
12/3/26	++++	..	..	..	2/17/28	+	2/20/28	++++	+++
12/14/26	++++	12/20/26	-	-	..	..	3/19/28	+++	-
12/29/26	++++	12/30/26	++	-	..	..	5/9/28	+	-
1/6/27	++++	1/6/27	+	-	5/12/28	+	5/14/28	+++	++
1/18/27	++++	1/20/27	-	-	..	..	6/27/28	++	-
2/12/27	++++	2/12/27	-	-	7/12/28	++	7/14/28	++++	+++
2/18/27	++++	2/21/27	++++	-	9/26/28	+	9/26/28	++	-
..	..	2/24/27	++++	-	..	..	10/1/28	++++	+++
..	..	2/28/27	+++	-	..	..	11/24/28	++	-
..	..	3/3/27	++	-	11/26/28	++	11/28/28	++++	+++
3/3/27	++++	3/5/27	++	-	1/30/29	++	2/1/29	++++	+++
..	..	3/11/27	+	-	7/3/29	+	7/5/29	+++	++
3/26/27	++++	4/24/27	+	-	..	..	7/9/29	Blood urea nitrogen 48.53	
4/25/27	++++	4/27/27	-	-	..	..	1/2/30	-	-
..	..	4/30/27	+++	-	..	..	1/9/30	+	-
..	..	5/3/27	-	-	1/6/30	++	1/30/30	++	-
5/6/27	++	5/9/27	-	-	1/27/30	++	5/8/30	++++	++
5/17/27	++	5/19/27	-	-	..	..	Died 7/26/30		
5/23/27	++	5/25/27	+++	++					

endothelial cells. A few free mononuclear cells are seen which are possibly of hematogenous origin, but nearly all the cells appear to be attached to the basement membrane and are therefore interpreted as endothelial in origin. Only an occasional erythrocyte is seen. The capillary basement membrane is thickened and often appears as a double layer. There is no change in the glomerular epithelium except some evidences of degeneration.

Nearly all the glomerular tufts show a structure similar to that shown in Fig. 3, but those tufts that have a hyaline appearance in the hematoxylin-cosin preparation have a somewhat different structure (Fig. 4). These show a similar increase of endothelial cells, but a very marked increase in the number of layers of the capillary basement membrane. The hyaline appearance of the tuft is clearly due to multiplication of the layers of the basement membrane. Tufts of this structure are no longer permeable to blood.

The renal lesion is therefore characterized by a diffuse proliferation of capillary endothelium and an increase in the thickness and number of layers of the capillary basement membrane. It differs from typical clinical glomerulonephritis in the absence of intracapillary hyaline fibers.<sup>1</sup> There is, however, a notable resemblance in glomerular structure to some human cases of "lipoid nephrosis of mixed type" or "nephritis with nephrotic tendency;"<sup>2</sup> but the similarity goes no further since there is practically no lipoid in the tubular epithelium and there was no edema.

It is highly probable that death was due to uremia. The blood urea nitrogen was 48.5 mgm. per 100 cc. seventeen months before death, but unfortunately it was not determined again. The glomerular obstruction and the tubular atrophy support this interpretation, and no other cause of death was found at postmortem.

In our judgment we have produced a form of chronic diffuse glomerulonephritis which resembles the "parenchymatous" type of the human disease, but does not correspond to any human lesion in all respects. The marked increase of capillary endothelial cells seems to justify the diagnosis of glomerulonephritis.

The lesion was produced by the repeated introduction of streptococci into the blood stream. Whether the bacterial bodies or their soluble toxins are responsible for the injury was not determined. The kidneys were at first resistant to injury, but later became highly

susceptible. This increased susceptibility of the glomeruli is probably merely a response of injured tissue to repeated irritation. It is not necessary to assume that allergic hypersensitiveness existed.

### SUMMARY

1. A form of chronic diffuse glomerulonephritis was produced in a monkey by repeated intravenous injections of streptococci over a period of four years.

2. The lesion is characterized histologically by marked increase in capillary endothelium and increase in the thickness and number of layers of the capillary basement membrane.

3. Histologically the glomerular lesion resembles human "lipoid nephrosis of mixed type," except that no fat is present.

### REFERENCES

1. McGregor, L. The finer histology of the normal glomerulus. *Am. J. Path.*, 1929, 5, 545.
2. Bell, E. T. Lipoid nephrosis. *Am. J. Path.*, 1929, 5, 587.

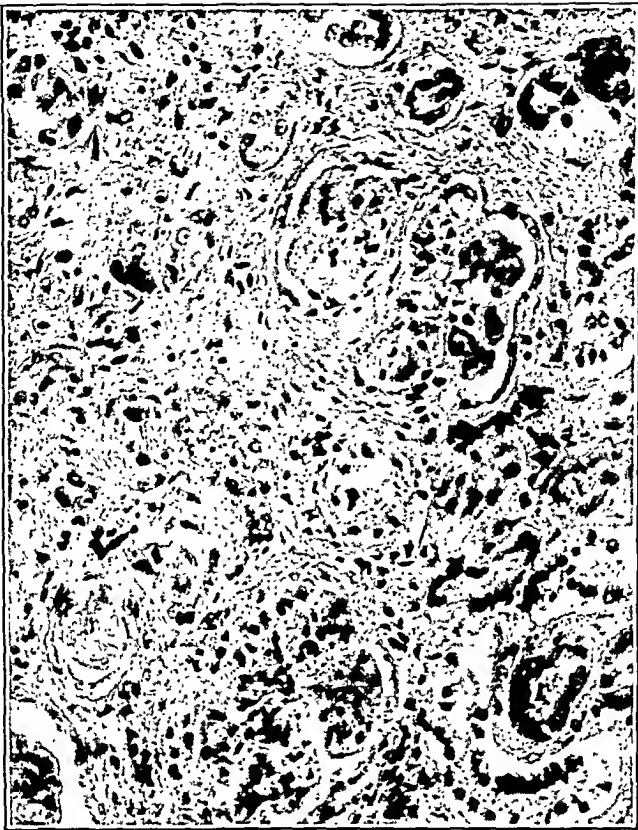
## DESCRIPTION OF PLATES

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### PLATE 10

FIG. 1. Area from the cortex showing moderate tubular atrophy. The glomerular lobules are fused to the capsular layer in some places and their capillaries are partially or completely closed. Iron-hematoxylin stain.

FIG. 2. Two lobules from a glomerulus shown in Fig. 1, under higher magnification. Note occlusion of the capillaries. Iron-hematoxylin stain.



1

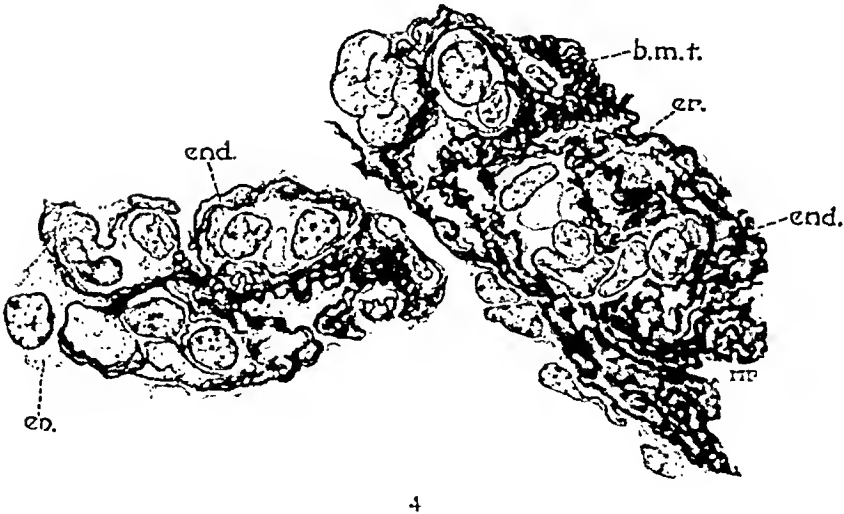
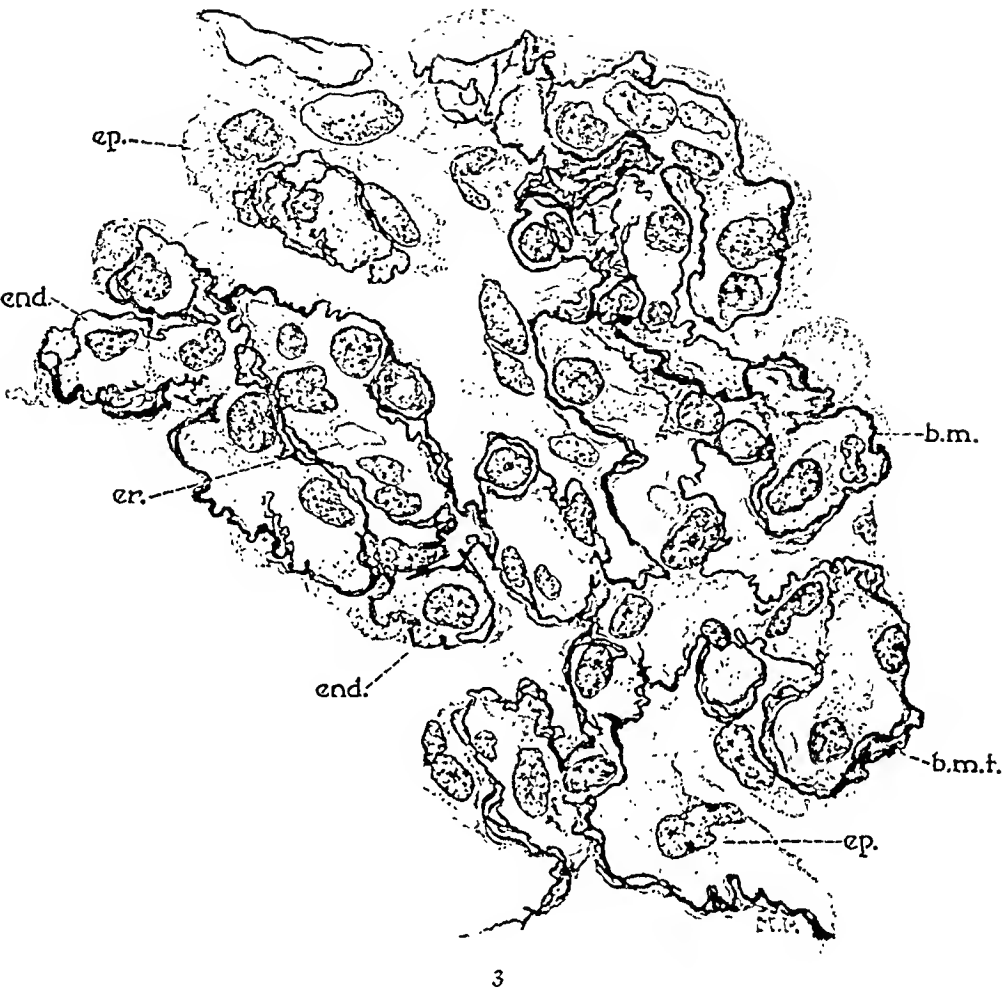


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## PLATE II

FIG. 3. Area from a glomerulus stained by McGregor's method (Heidenhain's modification of Mallory's anilin blue). Note marked increase of endothelial cells (*end.*). The glomerular epithelial cells (*ep.*) show no changes except evidences of degeneration. The capillary basement membrane (*b.m.*) is thicker than normal and is often represented by two or more layers (*b.m.t.*). (*Er.*) erythrocyte.

FIG. 4. Hyaline lobules from a glomerulus. Stained by McGregor's method. Lettered as in Fig. 3. Note the increase of endothelial cells and the multiplication of layers in the capillary basement membrane.







## A CASE OF HYPERNEPHROMA WITH TUMOR THROMBOSIS OF VENA CAVA AND HEART \*

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(From the Department of Pathology, Jewish Hospital of Brooklyn, Brooklyn, N. Y.)

In 1911 Pleasants,<sup>1</sup> in an excellent monograph on the subject of thrombosis of the vena cava, tabulated 29 cases collected from the literature in which the primary source of the neoplastic thrombosis was malignancy of the kidney. Since then other cases have been reported, and in 1925 Simpson<sup>2</sup> after reviewing the literature up to that year found 32 cases. The latter investigator justly points out the futility of attempting to separate the carcinomas from the sarcomas of the kidney in these instances because of the confusion in the terminology by the earlier writers. Intracardiac extension of the tumor thrombi occurred in 13 of these cases, 4 of which were definitely hypernephroma — the cases reported by Sternberg,<sup>3</sup> Wendell,<sup>4</sup> Weber,<sup>5</sup> and Jacobsen and Goodpasture<sup>6</sup> (see table).

Weber's case presented more extensive involvement than any of the others and resembles in many respects the case reported here. In this instance, as in the case described by Weber, the hypernephroma involved the left kidney and adrenal, forming a tumor thrombus which extended along both renal veins, completely occluding the vena cava and involving the iliac veins below, and the heart above.

### CASE REPORT

*Clinical History:* M. S., a white male, 55 years of age, was admitted to the Jewish Hospital of Brooklyn on the medical service of Dr. M. Rabinowitz, April 25, 1928.

*Present Illness:* Two weeks before admission the patient developed an abdominal swelling and edema of both lower extremities.

*Past History:* This is irrelevant except for an attack of lobar pneumonia eight weeks prior to admission. Following that attack, there appeared a swelling of the left lower extremity and a similar swelling of the right lower extremity. This condition subsided several weeks before admission.

*Family History:* Irrelevant.

*Physical Examination:* The patient appeared quite comfortable, although emaciated. The heart was negative except for the poor quality of its sounds. The lungs were dull to percussion over both bases, especially on the right side.

\* Received for publication November 3, 1930.

TABLE I

Cases of Malignant Tumors of the Kidney with Neoplastic Thrombosis of Inferior Vena Cava and Heart

Author	Age of patient years	Diagnosis	Involvement of iliac veins	Degree of involvement of inferior vena cava	Degree of involvement of heart
Barlow and Rose <sup>7</sup>	36	Carcinoma of right kidney	None	From renal veins to heart	Right atrium
Coyne and Troister <sup>8</sup>	45	Carcinoma of left kidney	None	From level of the fourth lumbar vertebra to heart	Right atrium
Engelken <sup>9</sup>	4	Teratoma of right kidney	None	From renal vein to heart	Right atrium
French <sup>10</sup>	45	Carcinoma of left kidney	None	From renal vein to heart	Right atrium into tricuspid valve
Gairdner and Coats <sup>11</sup>	53	Carcinoma of right kidney	None	Simple thrombus from iliac to renal veins and carcinoma- tous thrombus from renal vein to heart	Emboli in right ventricle
Jacobsen and Goodpasture <sup>6</sup>	63	Hypernephroma of left kidney	None	From iliac bifurcation to heart	Right atrium and ventricle
Judd and Scholl <sup>12</sup>	29	Adenocarcinoma of right kidney	None	From renal veins to heart	Right atrium
Judd and Scholl <sup>12</sup>	51	Carcinoma of one kidney	None	From renal vein to heart	Right atrium
Kluge <sup>13</sup>	74	Carcinoma of left kidney	None	From renal vein to heart	Right atrium and ventricle
Rosenstein <sup>14</sup>	6	Myosarcoma (cylindroma) of left kidney	None	From renal vein to heart	Right atrium
Sternberg <sup>3</sup>	60	Hypernephroma right kidney	None	From a point about 1.5 cm. above iliac veins to heart	Right atrium
Weber <sup>5</sup>	49	Hypernephroma involving both kidneys	Both veins thrombosed	Complete	Right atrium
Wendell <sup>4</sup>	?	"Epinephroid" carcinoma of right kidney	None	From iliac veins to heart	Right atrium
Wylar <sup>15</sup>	48	Carcinoma of left kidney	Both veins thrombosed	Complete	Right atrium and ventricle
Polayes and Taft	55	Hypernephroma left kidney, extension to the right kidney	Both iliac veins throm- bosed	Complete	Right atrium and ventricle

The liver was palpable three finger-breadths below the costal margin. In the lower left quadrant of the abdomen a mass 10 cm. in diameter was felt which was fixed posteriorly, slightly tender, smooth and firm on palpation. The upper and lower poles of this mass could not be palpated. The veins of the right side of the abdomen, as well as chest were quite distended. Those on the left side showed only slight distention. The right lower extremity was slightly edematous.

*Laboratory Data:*

Blood count: red blood cells 4,950,000 per cmm., white blood cells, 8,200 per cmm., polynuclear leucocytes 74 per cent and lymphocytes 26 per cent. The hemoglobin was 80 per cent (Dare).

Blood chemical analysis: Sugar 107 mg., urea nitrogen 17 mg., creatinine 1.9 mg., and uric acid 4.4 mg. per 100 cc.

Blood Wassermann and Kahn tests: negative.

Urinalysis: Albumin, a trace; sugar, negative; specific gravity, 1.004 to 1.022. Microscopic examination showed numerous red blood cells.

X-ray examination: Flat plate showed opacity not separated from kidney shadow suggestive of either a large or pathological kidney. The other kidney was found to be larger than the average normal kidney. Renal calculi were not visualized.

The chest plate showed a nodule in the left lung suggestive of a metastatic lesion.

Cystoscopic examination: negative.

Pyelogram: negative.

*Diagnosis:* Hypernephroma of left kidney with thrombosis of the renal vein and inferior vena cava.

The patient was transferred to the surgical service of Dr. J. Linder and a left nephrectomy was performed. The pathological report on the resected kidney was as follows:

*Macroscopic Examination:* The kidney measures 15 by 8 by 4 cm. The lower pole is the seat of a large nodular mass which invades about one-half of the kidney. The capsule strips with ease, except at those points where it is attached to the nodules of tumor tissue which project through the cortex. There are a few small cortical cysts on the surface. On section the major portion of the tumor is sharply circumscribed. It presents a honeycombed appearance and is made up of a mixture of bright yellow, brown, red and gray tissue.

Numerous smaller nodules of varying size are scattered throughout the rest of the organ (see Fig. 1). The renal vein contains a tumor growth which infiltrates its walls.

*Microscopic Examination:* The renal structure is distorted and compressed by the tumor tissue which invades it. Large areas are present showing pressure atrophy of renal parenchyma, the latter being characteristically sharply defined from the invading tumor.

The neoplastic structure is typically hypernephroma. It consists of cords of large transparent cells with foamy cytoplasm and small dark nuclei. Large blood vessels in a delicate supporting stroma separate cords of cells. Many of these vessels reach enormous size and some of them have ruptured so that extravasated blood is found at numerous points. Tumor thrombi occlude many of the large vessels. The wall of the renal vein is infiltrated with masses of neoplastic cells and its lumen is occupied by a solid plug of tumor tissue.

*Diagnosis:* Hypernephroma with infiltration and thrombosis of renal vein.

The patient was discharged after an uneventful postoperative course. About eight months later, however, he was readmitted to the hospital complaining of a recurrence of the abdominal swelling and extreme weakness.

On physical examination he showed the following findings. The lungs were flat to percussion and the breath sounds were absent. The abdomen was distended. A fluid wave and shifting dullness were easily demonstrable. The liver was found to be enlarged, hard and smooth. A small nodular area was palpable in midepigastrium.

*Diagnosis:* Malignant thrombosis of inferior vena cava with visceral metastasis and ascites.

A paracentesis performed soon after admission yielded 350 cc. of straw-colored fluid which was found to be sterile and to contain many large deeply staining cells with dividing nuclei and many signet ring forms strongly suggestive of malignancy. The patient failed rapidly and several days after admission lapsed into coma and died two days later.

#### AUTOPSY REPORT

The body is that of a well developed and undernourished white male, 55 years of age, with extreme cyanosis of the head and neck, and marked wasting of the panniculus and muscles.

*Peritoneal Cavity:* Moderately distended by a brownish yellow fluid.

*Pleural Cavity:* A small quantity of clear yellow fluid on each side, and firm bands between the right lower lobe and posterior wall of chest and diaphragm are present.

*Pericardial Cavity:* Markedly enlarged.

*Heart:* Moderate hypertrophy of myocardium in all chambers. The right atrium is markedly dilated and filled with a gray and red friable mass which occludes the right auriculoventricular orifice and extends into the right ventricle. This mass is continuous with a similar one in the inferior vena cava.

Microscopic examination shows edema of the myocardium. The intracardiac mass consists of tumor cells which are attached to the atrial wall and which form an integral part of a thrombus made up of definite layers of platelets and red cells held together by fibrin network.

*Inferior Vena Cava:* The vessel is dilated to about 5 cm. in diameter by a solid thrombus which extends downward to occlude the iliac veins on both sides, and which fills in the entire lumen of the cava involving the hepatic veins and extending to and continuous with the mass described in the heart (see Fig. 2). The microscopic examination of the thrombus shows a structure exactly like that found in the heart.

*Aorta:* Normal.

*Tracheobronchial Lymph Nodes:* Extensive metastatic involvement.

*Lungs:* Nodular thickenings throughout the parenchyma, made up of soft gray structure traversed by many blood channels. Healed tuberculous lesion in apex of left lung.

The microscopic examination shows nodules to consist of tumor cells, many of which are undergoing mitosis.

*Liver:* The liver weighs 1220 gm. Surface studded with numerous gray-brown nodules. Hepatic veins filled with gray-red thrombi extending to the main hepatic veins which are occluded by thrombotic masses continuous with that in the inferior vena cava.

Microscopic examination shows diffuse hemorrhages in the parenchyma, especially about the central veins. The main hepatic veins are occluded by platelet thrombi with a few malignant cells embedded in the peripheral portion of the mass, and infiltrating the intimal lining of the vessels.

*Spleen:* Nodular surface and gray capsule. Vessels occupied by gray-red thrombi.

Microscopic examination shows fibrosis of parenchyma and marked dilatation of veins with areas of diffuse extravasation of blood into the pulp. The thrombi show no malignant cells.

*Pancreas:* Normal.

*Gall Bladder:* Normal.

*Stomach:* Mucosa is hypertrophied and congested. An adenomatous polyp about 4 mm. in diameter is present, situated on the lesser curvature near the pylorus.

*Intestines and Mesentery:* Normal.

*Adrenals:* The left adrenal is about 6 cm. in diameter. The cortex is of a normal golden yellow color and the medulla of a gray granular structure with red mottling. The adrenal vein is occluded and lost in the tumor mass which extends from the medulla, involving the major portion of the left renal vein.

The right adrenal is normal.

Microscopic examination of the left adrenal shows complete replacement of the medulla and portions of the cortex by tumor cells. Some of the neoplastic cells seem to arise from the cortex. The right adrenal shows only a moderate amount of hydropic degeneration of the basal cells of the cortex.

*Right Kidney:* The remaining kidney measures 12 by 6 by 4 cm. The capsule strips easily leaving a fairly smooth surface except for a few cortical arteriosclerotic cysts. The normal markings between cortex and medulla are well preserved. The peripelvic tissue is infiltrated by cauliflower masses which extend into the distal portions of the pyramids at numerous points. These growths are continuous with the masses occluding the renal veins and thus with the main thrombus in the inferior vena cava.

Microscopic examination shows tumor tissue invading the renal parenchyma at the points described above. The renal vein is also occluded by a tumor thrombus.

*Right Ureter:* The walls are thickened by inflammatory infiltration, but no neoplastic cells can be found.

*Urinary Bladder:* Hypertrophy of the mucosa.

*Genitalia:* Normal.

*Bones:* The ribs, cranium, femora and tibiae are normal.

*Lymph Nodes:* Neoplastic involvement in the nodes about the bronchi, in the mesentery, at the site of the nephrectomy and in a few of the prevertebral nodes on the right side near the hilum of the right kidney.

*Brain and Meninges:* Edematous.

*Anatomical Diagnoses:* Hypernephroma of the left kidney and adrenal with involvement of the right kidney; tumor thrombus in the renal veins, entire inferior vena cava and some of its main tributaries (iliac and hepatic veins) extending into the right atrium and ventricle; lymph node and visceral metastases.

### SUMMARY

This is the fifth case of hypernephroma with malignant thrombosis by invasion of the inferior vena cava and heart. It is unique in its extent of involvement. The entire inferior vena cava and all its main tributaries (iliac, renal and hepatic veins) are occluded by a continuous malignant thrombus which extends into the right atrium, through the tricuspid valve into the right ventricle. The cardiac involvement here is even more extensive than in the case described by Weber in which the thrombus did not extend beyond the right atrium. Another interesting feature is the fact that in spite of the massive involvement of the inferior vena cava and the right kidney, the right adrenal gland remained singularly free of metastasis.

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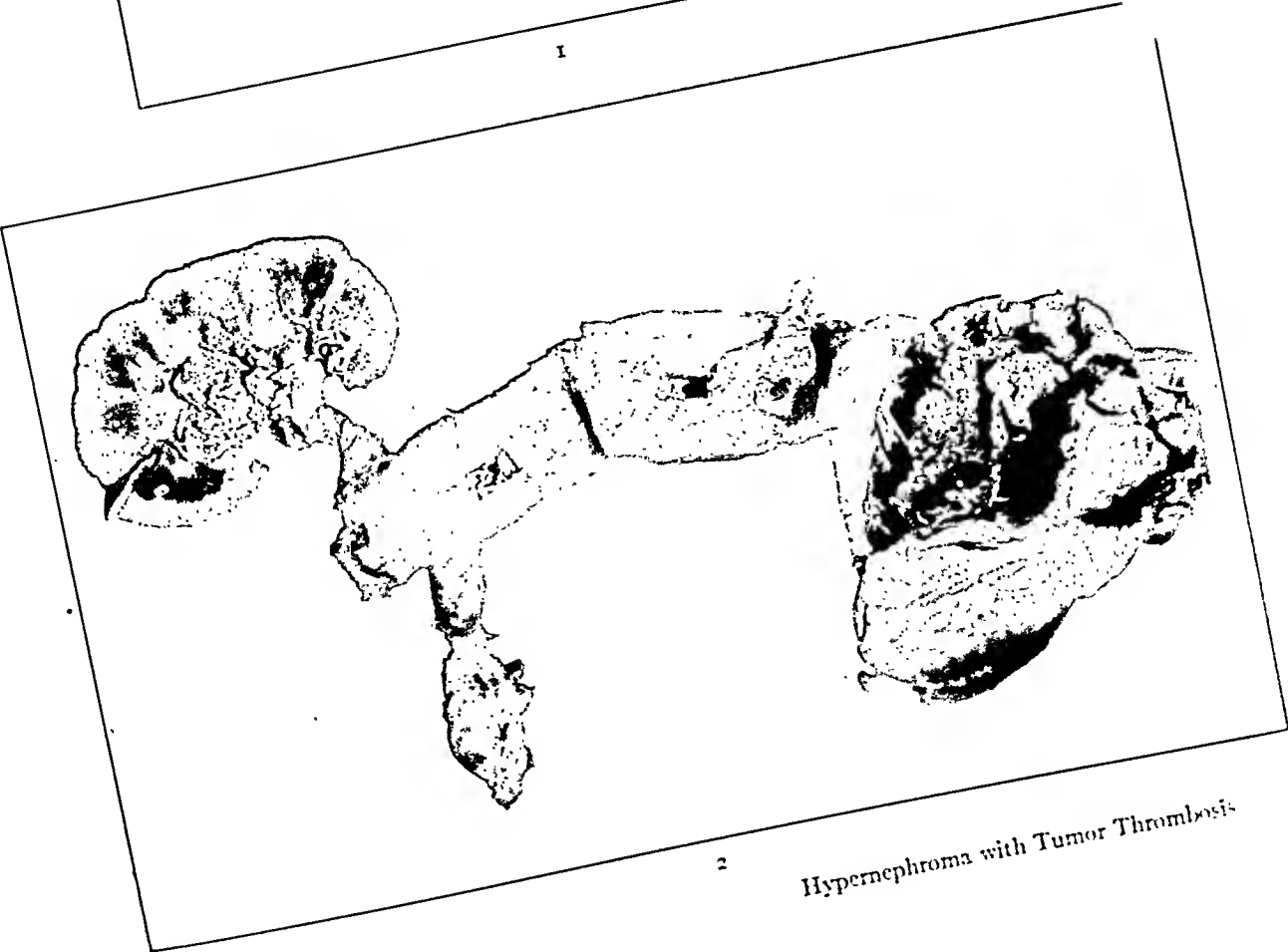
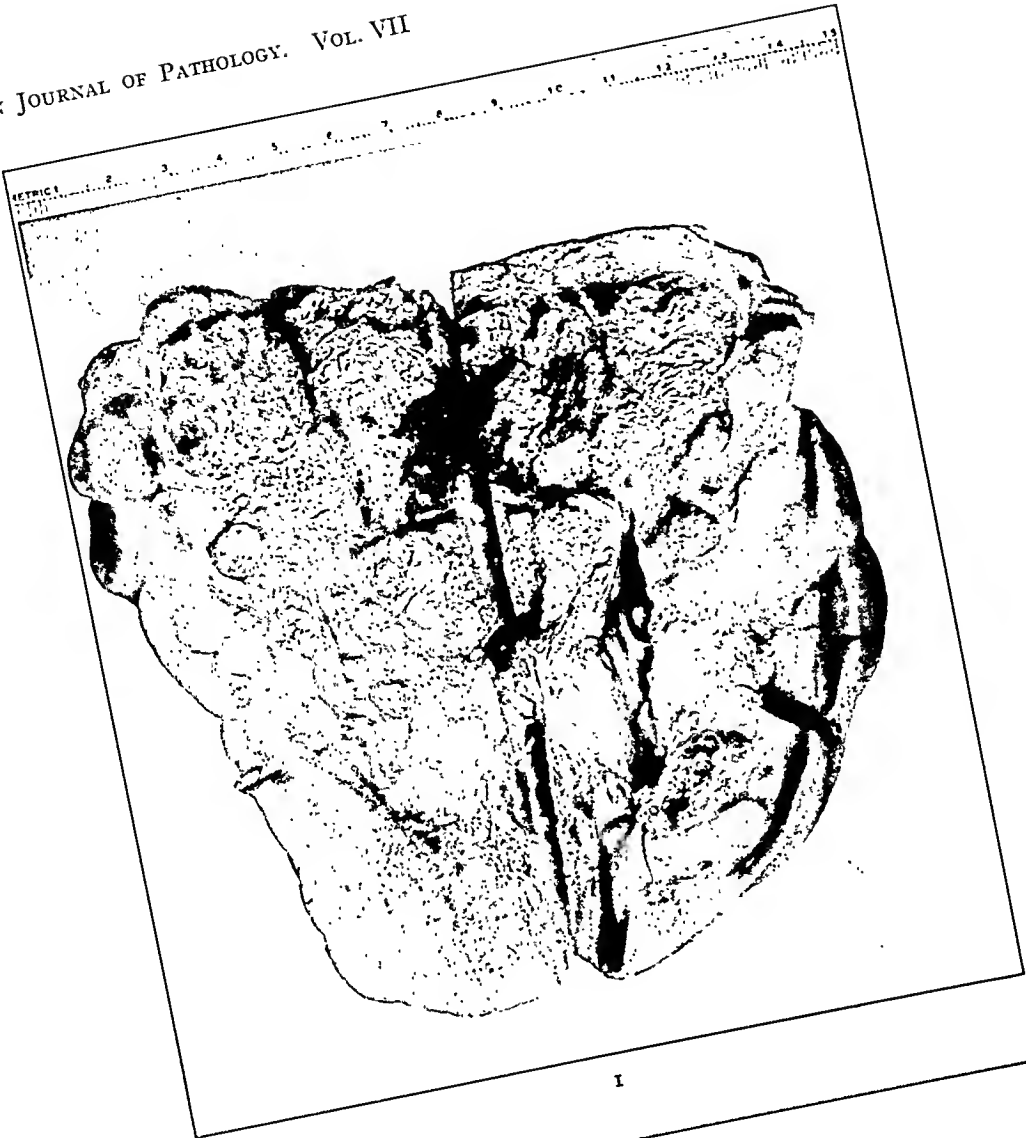
## DESCRIPTION OF PLATE

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### PLATE 12

FIG. 1. Left kidney (removed by nephrectomy) showing extensive involvement by hypernephroma. Note the sharp demarcation between tumor and renal tissue.

FIG. 2. Extension of hypernephroma with involvement of left adrenal and right kidney with neoplastic thrombosis in both renal veins, inferior vena cava, hepatic veins and heart. (Iliac veins have been severed.)



Hypernephroma with Tumor Thrombosis

ulation as to the relationship which may exist between the two substances. In the present case the homogeneous, eosinophilic substance, so widely deposited throughout the organs and tissues, has given characteristic staining reactions with iodine, Congo red and methyl-violet and has been considered true amyloid. Because of the diversity of opinion regarding the exact chemical nature of the Bence-Jones protein and amyloid, and the unsettled question as to their origin, it is impossible to decide whether we have present two chemically different proteins or whether the amyloid-like substance may be considered a "consolidation of the Bence-Jones protein," a suggestion offered by Wells<sup>6</sup> in regard to the case of Claus.

### SUMMARY

A case of multiple myeloma is reported with an associated extensive amyloidosis. Of interest, both clinically and pathologically, are the huge tumor-like masses which resulted from the deposition of amyloid in the striated muscles and about the shoulder joints. Worthy of note, also, is its presence in the spleen, kidneys, adrenals, gastro-intestinal tract, heart, pancreas, reproductive organs, sympathetic ganglia and adipose tissue and its absence from the parenchyma of the liver.

NOTE: I am indebted to Dr. W. W. Palmer for permission to transcribe the clinical records and to Mr. Alfred Reinberg for the drawings.

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surrounds many of the glands. About Brunner's glands the deposition is heavy and the amyloid forms wide trabeculae. It is present in the muscularis mucosae and in all the muscular coats of both stomach and intestine groups of muscle cells appear rose red in methyl violet preparations. Amyloid occurs also in the walls of the blood vessels in all the layers.

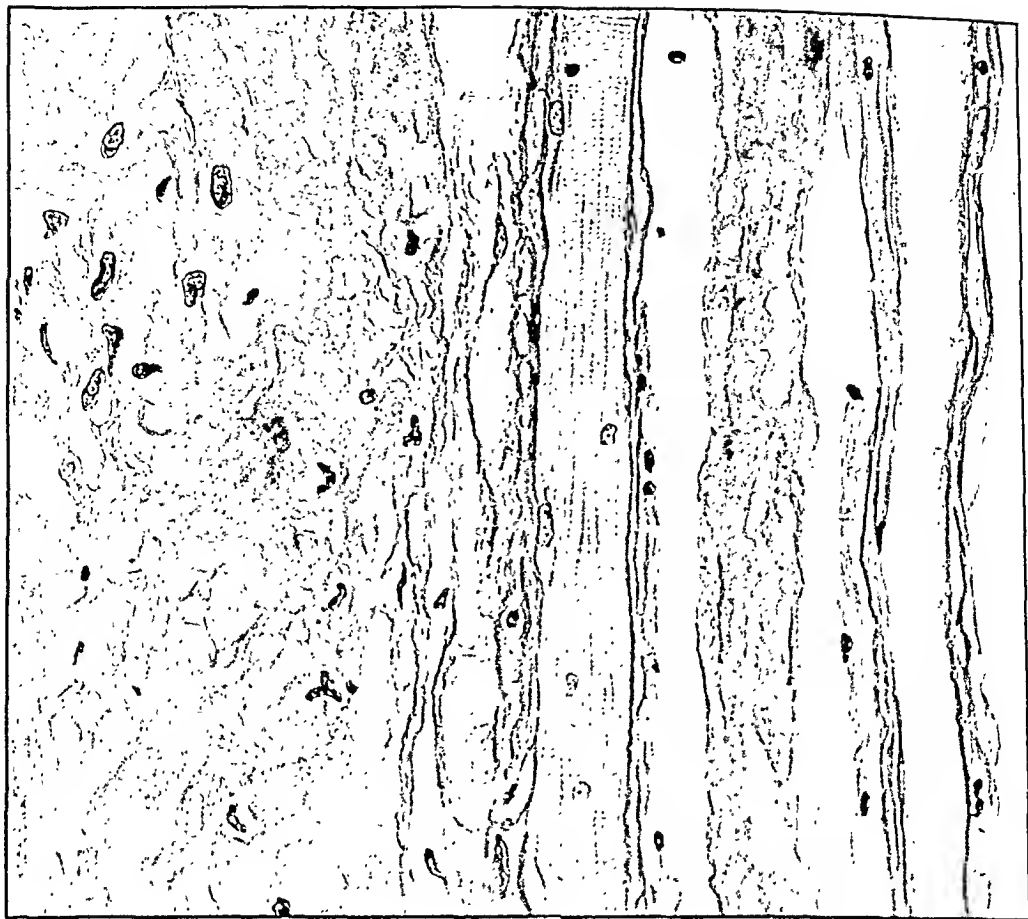
*Colon:* Amyloid is found only in the walls of the blood vessels of the submucosa and subserosa.

*Anatomical Diagnoses:* Multiple myeloma; amyloidosis of heart, spleen, liver, pancreas, adrenals, kidneys, testes, prostate, stomach, intestine, bone marrow, adipose tissue, sympathetic ganglia, shoulder joints and muscles of neck, shoulders, thorax, abdominal wall and thighs; infarct of heart; hydrothorax, bilateral; atelectasis of lungs, bilateral; cholelithiasis, calculus in gall-bladder; medial calcification of aorta; melanosis of colon; fibrous peritoneal adhesions.

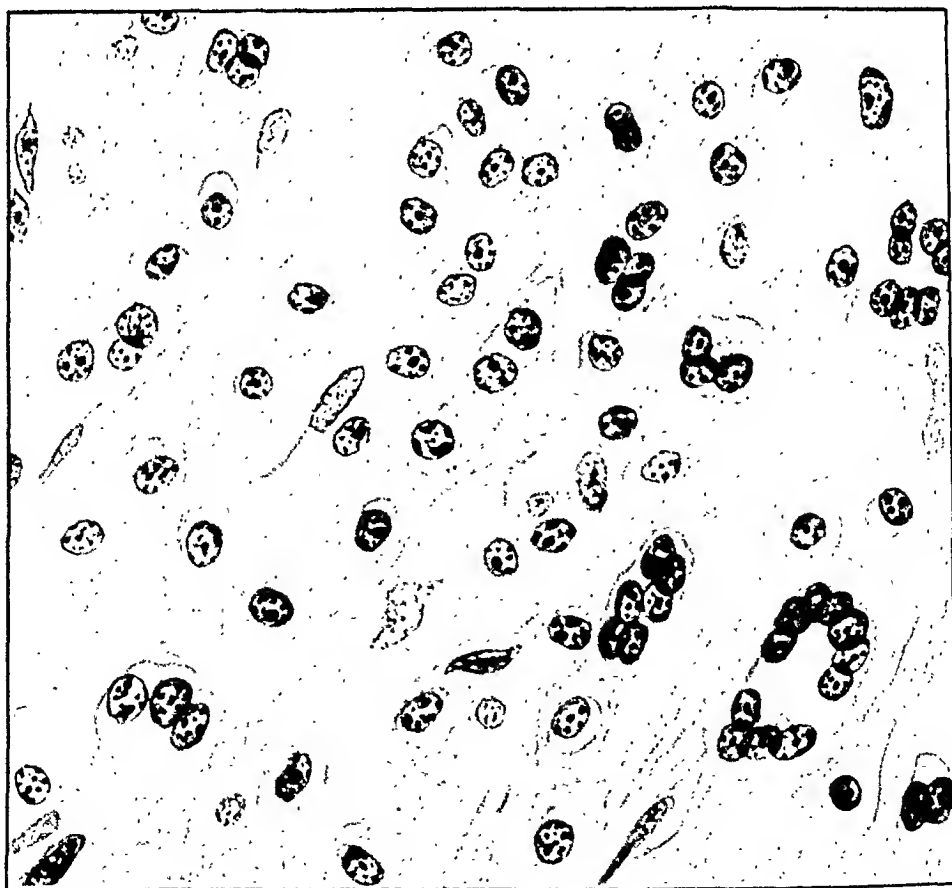
#### COMMENT

The case is one of generalized amyloidosis, associated with a widespread myeloma which involves, according to the pathological and X-ray findings, the spine, ribs, clavicles, humeri, femora, pelvic bones and skull. Microscopic examination shows the myeloma to be of the plasma cell type, in which the cells lie in a well developed fibrillar stroma. Of special interest are the sites of amyloid deposition with the formation of "amyloid tumors" about the shoulder joints and in the striated muscles of the abdominal wall and thighs. Undoubtedly the masses palpable in both antecubital fossae were also amyloid in nature, though this fact was not established at autopsy. Of the organs usually showing amyloid degeneration, the spleen, kidneys and adrenals in this case are affected but the liver is spared, except for the deposition of the substance in the walls of the hepatic vessels. Amyloid is abundant, however, in organs and tissues less commonly involved in the degenerative process and it appears in considerable quantities in the heart, gastro-intestinal tract, prostate and adipose tissue. Smaller depositions of it are present about the blood vessels of the testes, the periprostatic ganglia, the bone marrow and pancreas, and in the last organ a few of the lobules are infiltrated by it.

The presence of amyloid in cases of myeloma in which the Bence-Jones protein is an accompanying feature affords ground for spec-



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## DESCRIPTION OF PLATE

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### PLATE 153

FIG. 1. Plasma cell myeloma.

FIG. 2. Amyloid deposition in striated muscle.

# MELANOSIS MUCOSAE APPENDICIS VERMIFORMIS \*

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## REVIEW OF LITERATURE

Melanosis of the appendiceal mucosa has been studied hitherto only in connection with melanosis coli. The purpose of the present paper is to show the frequency of this condition in surgically removed appendices and to attempt to analyze its relation to other available data. Little has been added to the pathological anatomy and histology of melanosis coli since the thorough description of this condition by Pick<sup>1</sup> in 1911, who collected twelve cases from the literature, (one of Virchow's, one of C. J. Williams', one of G. N. Pitt's, one of Rolleston's, one reported by Grawitz, seven by Solger) and reported six additional cases, two of which were autopsied by Orth and one by Fraenkel.

Pick described granular brown pigment usually intracellular in round, oval, angular or irregularly shaped mononuclear cells about 14 microns in diameter, limited strictly to the mucosa and to the colon. Pigmentation was reported in the mucosa of the appendix in three of his own cases, in one of which there was distal obliteration. The earlier literature did not mention the appendix. He considered the pigment a melanin, and in 1930 with Brahn<sup>2</sup> reported further chemical examinations of pigment isolated from the melanotic mucosa which show that the pigment is a true melanin.

Simon<sup>3</sup> examined 77 appendices, 43 removed surgically and 34 at autopsy, finding pigment in the mucosa in 14. From his protocols this pigment appears to have been iron-free, intracellular in enlarged connective tissue or chromatophore-like cells situated in the interglandular stroma of the mucosa, and occasionally in the lymph follicles. He also cited four cases of "nodular and villous melanosis of the colon" in which the pigment contained iron, and hence were probably not true melanosis. Schmidt<sup>4</sup> examined 20 appendices removed at autopsy and found "hemosiderin" pigment in 15, but noted no melanin. Whether the methods used were adequate to





believed to be plasma cells which ranged from 7 to 20 microns in size. In 206 specimens of intestinal tissue there were 12 in which the colon was resected or examined in part in cases of chronic intestinal stasis. Eleven of these last were pigmented. Pigmentation of the appendix was noted specifically only in his Case 2. In the discussion of McFarland's presentation, Ewing<sup>10</sup> stated that he encountered pigmentation of the large intestine somewhat frequently and believed that a lesser degree of this condition was not so infrequent as the literature would indicate.

Lignac<sup>11</sup> found 7 cases in 600 autopsies and observed one other at laparotomy. The pigmentation was confined to the mucosa and mesocolic lymph glands, and was more intense in the cecum and rectum than in the colon or appendix. The microchemical reactions were those of melanin, except that only a brown coloration was produced by 1 per cent silver nitrate.

Dalldorf<sup>12</sup> reported 5 cases of melanosis coli. The pigment was bleached more slowly by antiformin than was that of the liver or heart, and was contained both in fixed and wandering cells. Attempts to produce melanosis coli in a dog experimentally were unsuccessful, but the experiment lasted only eleven weeks.

Lubarsch and Borchardt<sup>13</sup> noted 17 cases of melanosis coli in some 5,000 autopsies. The cases occurred chiefly in the older age groups. In 100 appendices they found only three examples, 16, 25, and 33 years of age, all with chronic appendicitis.

Stewart and Hickman<sup>14</sup> reported the incidence of melanosis coli in 670 autopsies including 100 cases of carcinoma of the colon. In 600 autopsies, including 30 cases of carcinoma, gross pigmentation was present in 67 (11.2 per cent) with the same preponderance in the higher age groups as reported by Henschen and Bergstrand. The 100 cases of carcinoma of the colon showed a much higher incidence of melanosis, 55 cases on gross examination and 13 more microscopically. Extension of pigmentation into the appendix was noted in some cases of melanosis coli, and they stated that melanosis was not infrequent in surgically removed appendices.

#### DESCRIPTION AND ANALYSIS OF MATERIAL

The material herein reported comprises 750 appendices examined in the course of the past three and a half years. As the condition apparently increased in frequency as greater familiarity with it was

distinguish these pigments is uncertain. Huecke <sup>5</sup> confined his discussion largely to the nature of the pigment of melanosis coli, finding it free from heavy metals, acid insoluble, bleached by peroxides, tinged blue by Nile blue after bleaching, and stained brown by silver nitrate. He considered it more closely related to the lipofuscins than to the true melanins. The pigment was contained in both fixed tissue and wandering cells.

Henschen and Bergstrand <sup>6</sup> analyzed 225 autopsies as to the presence, histological distribution, age and sex distribution, and relation to other factors of melanosis coli. In 5 of these 225 cases melanosis was definitely recognized grossly, and was found histologically in 65, or about 29 per cent. They found pigment also in the ileum and once in the mesocolic lymph glands. The appendix was missing in 3 cases, obliterated and pigment-free in 12 cases, pigmented alone in 10 cases, pigmented in association with colonic pigmentation in 25 cases, and unpigmented in the presence of cecal pigment in 15 of the 65 cases showing pigment in some part of the intestinal mucosa. In how many of the pigment-free cases the appendix was absent or obliterated is not stated. They found an association of melanosis with chronic constipation, and an increased prevalence in the higher age groups (35 of 85 cases over 60 years of age). The instances of isolated appendiceal melanosis were all in patent, dilated appendices.

Microchemical examinations showed that the pigment resembled that in the chorioid coat of the eye in many respects, but differed in others. They found the pigment in mononuclear cells about 15 microns in diameter which they considered as of macrophage type. The granules were at first small, later enlarging. Nuclear degeneration was noted, and instances of cells with much coarsely granular pigment and well preserved nuclei were interpreted as examples of secondary phagocytosis.

Niklas <sup>7</sup> reported two cases of colonic melanosis with intracellular and free crystalloid melanin-like pigment also in the mesocolic lymph glands. In one of these the appendix showed a wide-open lumen and a pigmented mucosa.

Bland-Sutton <sup>8</sup> observed melanosis of the mucosa in a surgical specimen removed for an annular carcinoma of the "iliac" colon.

McFarland <sup>9</sup> described melanosis coli similar to that described by Pick, and Henschen and Bergstrand. The pigment-carrying cells he

into five classes: (1) negative; (2) those showing only occasional pigment-laden cells in one or more cross-sections, designated as +; (3) those showing a few pigment cells as ++; (4) a moderate number as +++; and (5) those with heavy pigmentation which is often macroscopically evident as +++++. The rating was done on paraffin sections extracted first with 20 per cent sulphuric acid and then stained thirty seconds in 0.1 per cent toluidin blue. This method brings out the pigment quite conspicuously as green granules, and was selected for the survey of a large number of specimens as giving more histological detail than unstained sections, and also as applying two of the reactions for melanin.

The distribution according to age shows a slight increase in average age as the intensity of pigmentation increases (Table II).

TABLE II  
*Age Distribution by Decades, and Average Ages in Various Grades of Melanosis*

Grade	0-9	10-19	20-29	30-39	40-49	50-59	60-69	Age not given	Average age	Total No.
Negative.	1	24	213	155	50	19	4	18	30.7	484
+ .....	2	4	48	42	9	2	1	3	30.1	111
++ .....	0	4	24	36	7	4	0	9	32.2	84
+++ .....	0	2	16	21	9	3	0	3	32.8	54
++++ .....	0	0	0	0	0	0	0	2	34.2	17
Total ....	0	1	2	9	3	0	0	18		
3										
35										
303										
263										
78										
28										
5										
35										
30.99										
750										

Adding those showing traces of pigmentation to those showing none forms a group showing about two years lower average age than those showing pigmentation rated at ++ or more (30.6 years against 32.6).

Grouping the cases according to race and sex is not especially valuable, as 665 of the total 750 are white males, corresponding to the predominance of this group among the beneficiaries of the Public Health Service. The incidence in this group is not significantly different from that in the entire series, and the groups of white females and of all non-white are so small that the somewhat greater percentage variations shown are not particularly significant (Table III).

Dividing the cases according to occupational groups showed no great differences in the five groups used except that the group com-

acquired, fresh sections were prepared of all negative cases within the last three months of the work and reexamined. It was found that no significant variation in frequency had actually occurred.

TABLE I

*Variation in Frequency of Melanosis in 750 Appendices*

Count	1st 100	2nd 100	3rd 100	4th 100	5th 100	6th 100	7th 100	last 50	Total per cent
Per cent . . . . .	35	35	32	44	51	25	31	26	35.5

The histological picture corresponds well with that described by Pick. The pigment is pale yellow to deep brown in color, finely to coarsely granular in form, insoluble after formalin fixation in alcohol, acetone, benzol, xylol, hydrochloric and sulphuric acids. It does not react for free iron by the ferrocyanide procedure. It tinges orange-red with safranin and blue-green to yellowish green with methylene blue or toluidin blue, and remains unstained by basic fuchsin. In view of the extensive investigation of the chemical nature of this pigment by various authors, further tests of its nature were not made, the above being deemed sufficient for its identification as a melanin.

This pigment is usually contained in cells which are generally confined strictly to the stroma of the mucosa, only rarely being found in the periphery of the lymph follicles. The cells are large and round with broad cytoplasm, or stellate, fusiform or angular with narrower cytoplasm, but without the long slender processes seen in the chromatophores of the iris. The nuclei of both types, where distinguishable, are round or oval, leptochromatic in structure, with narrow nuclear membrane, small nucleoli and small chromatin granules.

In several cases there was an accumulation of large round mononuclear cells with leptochromatic vesicular nuclei just beneath the surface epithelium between the mouths of the glands. These cells contained many fine acid-insoluble granules which were not visibly colored and which stained pale blue with toluidin after two hours' extraction with 20 per cent sulphuric acid. As such cases often contain typical pigment in the deeper layers of the mucosa these granules are regarded as prophaes of typical melanin.

For the purposes of correlation with various other factors of different grades of pigmentation the cases have been arbitrarily divided



prising seamen, marine firemen and other subordinate seafaring personnel shows a somewhat lower total incidence of pigmentation than the other groups (Table IV), and the handworkers ashore a higher percentage.

TABLE III

*Distribution of Melanosis by Sex and Race*

Sex and race	Negative		+		++		+++		++++		Total No.
	No.	%	No.	%	No.	%	No.	%	No.	%	
White males....	425	63.9	103	15.5	75	11.3	48	7.2	14	2.1	665
White females..	26	76	3	9	2	6	2	6	1	3	34
All non-white...	27	67	4	10	4	10	3	5	2	3	40
Total * .....	484	64.5	111	14.8	84	11.2	54	7.2	17	2.3	750

\* 11 cases in which race or sex and race were not stated are included in this group.

On grouping the cases according to concurrent histological diagnoses it is found that with acute inflammation or obliteration the incidence of pigmentation is markedly reduced, with subacute inflammation less so. For the purposes of this study acute appendicitis comprises those cases showing acute ulceration of the mucosa or diffuse polymorphonuclear infiltration of the outer parts of the appendiceal wall, hemorrhagic necrosis, or in a few cases localized polymorphonuclear infiltration of the mucosa. Subacute appendicitis comprises cases showing notable eosinophil and lymphoid cell infiltration in the outer layers, granulating ulcers in the mucosa, organizing exudates in the serosa and mesenteric fat, or pus-filled glands in the mucosa. Chronic appendicitis is taken to comprise appendices showing more or less scarring, atrophy or hyperplasia of lymph follicles, noteworthy eosinophilic infiltration in the mucosa, and adhesions or obliteration. Some appendices originally classed as "normal" were placed in this group, as it was found that appendices removed at operations for other conditions where no symptoms of appendicitis were noted were not to be distinguished from those removed in "interval" operations for chronic appendicitis in many cases.

Bearing in mind the association of melanosis coli with chronic constipation noted in the literature, it was thought that the presence of fibrous adhesions might influence the incidence of melanosis in the appendix by producing local stasis. Adhesions were reported by the

TABLE IV  
*Occupational Distribution of Melanos*

Number  Percent  Percent	Seafaring groups										"Shore" groups										Total 750									
	Officers, Petty officers = 82					Seamen, firemen, etc. = 370					Professional, clerical, etc. = 43					Laborers, mechanics, housewives, hand workers, = 199					No occupation or not stated = 56									
	-	+	++	+++	++++	-	+	++	+++	++++	-	+	++	+++	++++	-	+	++	+++	++++	-	+	++	+++	++++	-	+	++	+++	++++
52	13	6	9	2	251	54	35	24	6	26	9	4	4	0	122	25	32	13	7	33	10	7	4	2	484	111	84	54	17	
63	16	7	11	2	68	15	9	6	2	60	21	9	9	0	61	13	16	7	4	59	18	12	7	4	64	15	11	7	2	
Percent	79		21		82		18		81		19		74		26		77		23		78		22							

operating surgeons in 158 cases, of which 29 were completely or partly obliterated. Three of these 29 and 10 others showed subacute appendicitis, and 5 showed acute appendicitis. The group classed as "chronic without obliteration" showed no appreciable influence of the presence of adhesions (Table VI).

TABLE V  
*Relation of Melanosis to Acute and Chronic Appendicitis*

Grade	Acute		Subacute		Chronic without obliteration		Chronic distal $\frac{1}{2}$ obliterated		Chronic distal $\frac{3}{4}$ obliterated		Chronic complete obliteration		All appendiccs*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Negative. ....	130	91.5	79	71.8	220	51.9	17	59-	4	0	26	93-	484	64.5
+ .....	6	4.2	16	14.5	82	19.3	4	14-	3	0	0	0	111	14.8
++ .....	4	2.8	6	5.4	68	16.0	5	17+	1	0	0	0	84	11.2
+++ .....	2	1.4	9	8.2	40	9.4	1	3+	1	0	1	4-	54	7.2
++++ .....	0	0	0	0	14	3.3	2	7-	0	0	1	4-	17	2.3
Total .....	142		110		424		29		9		28		750*	

\*8 appendices which are included in the totals do not appear elsewhere in this table, their diagnoses being tuberculosis in 4, carcinoid in 2, adenocarcinoma in 1 and autolysis in 1.

TABLE VI  
*Relation of Melanosis to Adhesions and to Lymph Follicle Changes*

Grade	All adhesions		Adhesions, chronic without obliteration		Hyperplasia of lymph follicles		Partial atrophy, partial hyperplasia		Atrophy of lymph follicles	
	No.	%	No.	%	No.	%	No.	%	No.	%
Negative..	93	60.1	55	50.5	73	55.3	35	53.8	76	50.7
+ .....	23	14.6	19	17.4	27	20.5	11	16.9	26	17.3
++ .....	23	14.6	21	19.3	19	14.4	10	15.4	29	19.3
+++ ....	15	9.5	11	10.1	10	7.6	8	12.3	14	9.3
++++ ...	4	2.5	3	2.8	3	2.2	1	1.5	5	3.3
Total ....	158		109		132		65		150	

The degree of atrophy or hypertrophy and hyperplasia of the lymph follicles showed little influence on the incidence of melanosis, there being possibly a slightly higher incidence of pigmentation in the group with atrophied follicles (Table VI). Only the group classed as "chronic without obliteration" was used in this last tabulation, in order to avoid the influence of obliteration and of subacute and acute inflammation already demonstrated.



The group of "chronic appendicitis without obliteration" was next analyzed as to the relation of the incidence of melanosis to the duration of symptoms of chronic appendicitis. The group designated "no symptoms" includes the appendices removed at herniotomies, pelvic operations, and at laparotomies for other causes in instances where there had been no symptoms attributed to the appendix. The group designated "0-6 days" includes the cases in which no specific duration of symptoms was given, but the case designated clinically as "acute appendicitis," while similarly diagnosed "subacute appendicitis" is included in the group 7-29 days. Cases diagnosed clinically as "chronic with unspecified duration," or "duration of years" are grouped with those of specified duration of over two years (Table VII).

TABLE VII  
*Relation of Duration of Symptoms of Appendicitis to the Incidence of Melanosis in the Chronic, Non-Obliterative Group*

Grade	No symptoms		0-6 days		7-29 days		1-6 months		7 mos. - 2 yrs.		2 years, chronic		Total		Average of duration specified in yr. mos. days
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Total ..	39		30		45		96		93		114		417		
Negative	20	51	15	50	23	51	57	59	48	52	55	48	218	1	4 19
+	12	31	7	23	11	25	12	13	17	18	21	18	80	2	3 20
++	5	13	4	13	6	13	20	21	14	15	19	17	68	1	11 21
+++	2	5	4	13	3	7	4	4	10	11	14	12	37	2	1 16
++++	0	0	0	0	2	4	3	3	4	4	5	4	14	2	4 16

While the various symptom-duration groups shown in Table VII show no significant variation among each other or with the "no symptoms" group in the total percentage of positives, there seems to be a significant progressive increase in proportion of the grades of pigmentation greater than traces (+), and the average duration of symptoms of chronic appendicitis appears to be significantly greater in the pigmented cases than in the non-pigmented. As Henschen and Bergstrand noted dilatation in melanotic appendices, and assigned "local constipation" as the cause of isolated degree and incidence of melanosis with the presence or absence of dilatation. Five hundred and ninety-five of the 750 appendices of the series were received fixed in an unopened state, and were considered suitable for the estimation of dilatation. This was esti-

mated on the usual cross-sections, dilatation of one, two or all of three sections being recorded. The results for all appendices appear in Table VIII, those for appendices without acute or subacute inflammation or obliteration in Table IX. On calculating percentage distribution of the different grades of melanosis between contracted and partially or completely dilated appendices, it appears that pigmentation is distinctly more frequent in the latter group (Table X).

TABLE VIII

*Degree of Melanosis in Contracted and Dilated Appendices*

Dilatation	—	+	++	+++	++++	Total
Lumen small .....	231	32	35	14	3	315
Dilated 1 level .....	63	28	14	7	1	113
" 2 levels .....	56	20	7	4	3	90
" 3 levels .....	37	10	10	13	7	77
Total .....	387	90	66	38	14	595

TABLE IX

*Degree of Melanosis in Contracted and Dilated Appendices  
(Chronic Appendicitis Without Obliteration)*

Dilatation	—	+	++	+++	++++	Total
Lumen small .....	96	17	27	9	2	151
Dilated 1 level .....	29	22	12	5	1	69
" 2 levels .....	29	15	6	4	2	56
" 3 levels .....	20	9	8	10	7	54
Total .....	174	63	53	28	12	330

TABLE X

*Percentage Distribution of Varying Grades of Melanosis in Contracted and Dilated Appendices (Chronic Appendicitis Without Obliteration)*

Degree of melanosis	Lumen small		Lumen dilated		Total No.
	No.	%	No.	%	
Negative.....	96	64	78	44	174
+ .....	17	11	46	26	63
++ .....	27	18	26	15	53
+++ .....	9	6	19	11	28
++++ .....	2	1	10	5	12
Total .....	151		179		330

# DISCUSSION AND CONCLUSIONS

Melanosis of the appendix resembles essentially the melanosis coli described by Pick and others, and probably often forms a part of that condition. The pigment is probably formed first as fine uncolored granules in large mononuclear cells immediately beneath the surface epithelium. The pigment-carrying cells appear to be of macrophage and fibroblast types.

The average age of patients with pigmented appendices is slightly greater than with no pigment. No especially significant differences in incidence were found on segregation according to race, sex or occupation.

The great diminution in frequency of melanosis in acute and obliterative appendicitides, while undoubtedly due in part to mucosal destruction is probably not due entirely to that factor, as non-obliterate, non-ulcerative subacute appendicitis shows a similar though less marked decrease in incidence.

Adhesions have no noteworthy influence on the incidence of melanosis, atrophy of lymph follicles is possibly associated with a slight increase, and dilatation is quite definitely correlated with an increased frequency and probably higher grade of melanosis. The duration of symptoms of chronic appendicitis appears to be definitely greater in melanotic than in non-melanotic appendices.

It is noteworthy that the frequency of melanosis here reported is greater than in any published reports on melanosis coli, and the association with chronic appendicitis with dilatation and of longer than average duration may indicate that such chronic appendicitis provides favorable conditions for increased deposition of the pigment.

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# MAST MYELOCYTE LEUKEMIA IN A CAT \*

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Mast myelocyte leukemia has been reported in man by Lazarus,<sup>1</sup> Joachim,<sup>2</sup> Tomaszewski,<sup>3</sup> DiGuglielmo,<sup>4</sup> Vasaturo,<sup>5</sup> Sabrazès,<sup>6</sup> Massa and Marinoni,<sup>7</sup> and Tinti,<sup>8</sup> counting as such only cases showing mast cell percentages above 20 per cent. This subvariety of myelogenous leukemia was so named by Joachim, who saw two cases. Vasaturo refers also to a case of Hubertin's showing 23 per cent mast cells and to a case of Sabrazès in 1924, the original reports of which could not be found.

TABLE I

*Reported Cases of Myelocyte Leukemia in Man*

Author	Date	White corpuscles	Mast cell percentage
Lazarus .....	1901	..	47 later 3.7
Joachim .....	1906	189,000-168,800	53.6 "
Joachim .....	1906	237,000-117,000	56.4 "
Tomaszewski .....	1911	267,400-130,000	28 "
DiGuglielmo .....	1925	..	40 "
Vasaturo .....	1925	550,000	55
Sabrazès .....	1926	80,000	22
Massa and Marinoni .....	1926	138,100	30.9
Tinti .....	1926	120,000	35

Autopsy findings have been reported only in Joachim's second case which showed in brief:

Bilateral serosanguinous pleural exudate, with lesser and more purely serous pericardial and peritoneal fluid accumulations; moderate enlargement of mediastinal, retroperitoneal, omental, cervical and inguinal lymph glands, most marked in the last; enlargement of the spleen to 2100 gm. with gray-white subcapsular foci of necrosis, and gray-white strands of leukemic infiltration in the red pulp; enlargement of the liver to 5400 gm. with pale color, indistinct lobulation with gray-white peripheries; and red to light gray-red femoral bone marrow. Marrow smears from various locations showed predominance of mast cells. Histologically the liver showed

periportal and interlobular leukemic infiltrations consisting chiefly of mast cells, but including also numbers of neutrophils, eosinophils and lymphocytes, and marked dilatation of the intralobular capillaries. These contained many highly vacuolated large mononuclear cells, red and white corpuscles, with predominance of mast cells among the latter. Numerous mitoses were seen in the infiltrated areas, presumably in mast cells, the high degree of water solubility of the specific granulations when satisfactory nuclear stains were used rendering this uncertain. Necroses containing mast granules or nuclear debris, hemorrhages and irregularly distributed leukemic infiltrations were seen in the spleen; leukemic infiltrations occurred also in the renal cortex and in lymph glands.

Joachim was inclined to attribute the splenic necroses to the intensive roentgen ray therapy, and commented on the relatively smaller decrease of absolute numbers of circulating mast cells under X-ray treatment.

Leukemia in mammals was first reported by Leisering in 1858 (Folke Henschen<sup>9</sup>) in the horse. Cases have been reported since, most frequently in dogs, less often in horses, oxen, swine, mice, cats, rarely in goats and sheep, once in an elephant (Folke Henschen,<sup>9</sup> Hirschfeld,<sup>10</sup> Hutyra and Marek<sup>11</sup>), and have more often been of lymphatic type. In fact Jolly<sup>12</sup> stated that myelocythemia was unknown in the higher animals, except that certain older reports mentioned enormous splenomegaly and pyoid marrow, but without adequate blood examination. Hirschfeld, however, refers to cases of myelogenous leukemia in the dog (Lüdke in 1910), and in cattle (Knuth and Volkmann, Du Toit), and Julliard<sup>13</sup> stated that myelogenous leukemia was the more frequent form in animals.

In cats, reported cases of leukemia have been extremely rare. At the end of Siedamgrotzky's<sup>14</sup> detailed presentation of the first reported case of leukemia in the dog there is a four-line note: "Also in a cat which died of internal hemorrhage there was found a low grade leukaemia whose anatomical expression lay in a significant hyperplasia of the lymph glands and of the spleen (to twice normal size)." This case would appear to have been of lymphatic type. Nocard's report<sup>15</sup> refers to Siedamgrotzky's case and not to an original observation. In 1889 Sommer (Hutyra and Marek<sup>11</sup>) collected forty-six cases of leukemia in animals, among which were two in cats. DeDoës<sup>16</sup> reported a case of pseudoleukemia in a cat.

Tellmann's case<sup>17</sup> showed twelve red cells to one white in the peripheral blood, which with the given red cell count of 300,000 per cmm. would indicate a white count of about 25,000 per cmm. The white cells were chiefly "multinuclear" and some small mononuclears of the hyaline type and numerous "embryonal" stages of red corpuscles were present. The autopsy revealed an enlarged, firm, dark red spleen with prominent malpighian corpuscles, an enlarged, soft and friable, grayish yellow anemic liver with petechial subcapsular hemorrhages on the upper surface and indistinct lobular markings, enlarged (bean-size) soft grayish white lymph glands, dark red gelatinous marrow in the long bones and many small white subpleural nodules in the lungs. Tellmann's diagnosis of leukemia of mixed lymphatic, splenic and myelogenous form appears highly questionable, the findings suggesting rather a hyperregenerative, megaloblastic anemia.

In an annual report of animal autopsies Joest<sup>18</sup> includes the diagnosis of splenic leukemia in a cat. Ball<sup>19</sup> in a general paper on leukemias, noted the occurrence of myelogenous leukemia in the cat, but gave no case report or reference to the literature. Ball and Auger<sup>20</sup> diagnosed lymphatic leukemia in a cat dying after some months of progressive emaciation and loss of strength. The only gross post-mortem finding was an enlarged (42 gm.) (normal 4 to 6 gm.) bluish red spleen with enlarged splenic corpuscles. Histologically there was hyperplasia of the lymphoid tissue of the enlarged malpighian corpuscles and hyperplasia of the reticular tissue of the hypertrophied pulp, but lymphocyte infiltration of the latter was not reported. Histological examination was apparently limited to the spleen. The blood showed "an intense anemia with considerable mononucleosis and inversion of the leucocytic formula." The reported data appear rather inadequate to establish the diagnosis.

Ball (Pinvidic<sup>21</sup>) considered leukemias as relatively frequent in dogs and cats, less frequent in horses, oxen, swine and birds, and said that they occurred in all forms in cats. Didier<sup>22</sup> (cited after Pinvidic) reported lymphatic leukemia in a cat, in which the liver was enlarged from the normal of 80 to 90 gm. to 480 gm., and histologically contained numerous lymphocytes in its capillaries and showed extensive areas of lymphoid infiltration. Pinvidic<sup>21</sup> diagnosed lymphatic leukemia at autopsy in a 5 year old cat. The spleen was enlarged to 38 gm., soft, bluish red in color, with tense capsule

## CHRONIC TYPHOID CHOLECYSTITIS \*

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### INTRODUCTION

A series of seven cholecystectomies in known typhoid carriers has afforded us interesting material for the study of chronic typhoidal cholecystitis. The duration of the carrier state in these patients varied from three months to thirty years and all but one had been responsible for the infection of other individuals. All of the patients presented symptoms suggestive of either gall-stones or cholecystitis, but in none were the symptoms of sufficient intensity to warrant an operation.

### REVIEW OF THE LITERATURE

Although Gilbert and Girode<sup>1</sup> noted the infection of the gall-bladder during the course of typhoid fever as early as 1890, it was not until Chiari's extensive investigation of twenty-two cases in 1894<sup>2</sup> that the frequency of typhoid infection of the biliary tract in the course of typhoid was recognized. Since then an immense literature has developed upon the subject, much of which is summarized by Garbat<sup>3</sup> and by Gay<sup>4</sup> in their extensive monographs. The great majority of these publications, however, are concerned with the bacteriological or epidemiological features of the condition and in an extensive review of these publications we have been able to find only three in which the histological picture was adequately described.

Chiari, in his original paper, found typhoid bacilli in the gall-bladder usually in pure culture, in nineteen of his twenty-two cases. Thirteen of these showed inflammatory changes, limited in all but one case (and that showed coinfection with staphylococcus aureus) to the mucosa, which showed more or less dense leukocytic infiltration, with hyperemia and edema. In only one case was there necrosis.

In 1913 Bindseil<sup>5</sup> described a single case as follows: "The mucosa was thickened, the folds thick and prominent because of the inflam-

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TABLE II

*Occurrence of Leukemia in Cats*

Author	Year	Form	Spleen	Lymph glands	Marrow	Blood	Remarks
Siedamgrotzky.....	1871	lymphatic?	twice normal	enlarged	..	..	..
Sommer .....	1889	..	..	..	..	..	collected 2 cases from literature
de Doës.....	1890	pseudoleukemia	..	..	..	normal?	..
Lellmann .....	1896	mixed	enlarged	enlarged	dark red	300,000 rbc 25,000 wbc	validity dubious
Joest.....	1905	splenic	..	..	..	..	no data
Ball .....	1912	myelogenous	..	..	..	..	no data
Ball and Auger ....	1924	lymphatic	42 gm.	not enlarged	..	anemia mononucleosis	validity not certain
Didier .....	1925	lymphatic	..	..	..	..	leukemic infiltration of liver
Pinvidic .....	1926	lymphatic	38 gm.	..	..	mobilization in veins	leukemic infiltration of spleen
Lund .....	1927	lymphatic	..	..	..	..	no specific data as to cats
Lund .....	1927	myelogenous neurophil and eosinophil	enlarged	slightly enlarged	gray-yellow	..	no specific data as to cats
Lille .....	1931	mast myelocytic	200-300 gm.	not enlarged	..	mobilization in veins	..

and enlarged splenic corpuscles. The liver weighed 150 gm., was orange-red in color and flecked with yellowish necrotic foci a few millimeters in diameter. Lymph glands were not mentioned. Histological examination was apparently limited to the spleen. The splenic pulp contained numerous lymphocytes and small hemorrhages with marginal siderosis, the splenic corpuscles were enlarged, rather poorly defined and typically lymphoid in structure, and the blood in the veins contained numerous lymphocytes.

Lund<sup>23</sup> included two cats in a report of leukemia in thirty-four mammals (twenty-five lymphatic and nine myelogenous). One of the cats presented the lymphatic form, the other the myelogenous. General descriptions of the gross and minute pathology of each form were given, without case protocols. The lymphatic form was described as it occurred in swine. The myelogenous type presented marked splenomegaly, relatively slight lymphadenopathy, grayish or greenish yellow bone marrow and grossly evident leukemic infiltrations of liver and kidneys. Histologically there was generalized proliferation and dissemination of myeloid tissue which was composed chiefly of neutrophilic and eosinophilic myelocytes, myeloblasts, some normoblasts, megaloblasts and megakaryocytes.

Julliard<sup>13</sup> considered myeloid leukemia more frequent than lymphatic in animals, and noted its occurrence in the cat, but no specific case report was mentioned in Schmidt-Hoensdorf's review.<sup>13</sup>

### CASE REPORT

In our own case the animal was an apparently healthy cat weighing 2500 gm. which was being used in pharmacological studies by Dr. M. I. Smith of the National Institute of Health. The animal died unexpectedly about two hours after the intraperitoneal injection of paracresyl phosphate (2.5 gm. in 25 cc. olive oil) and was autopsied to ascertain whether death had been due to hemorrhage following the injection. Dr. Smith found the spleen enormously enlarged, filling the whole front and left side of the abdomen, measuring about 28 by 10 by 2 cm. or possibly 15 per cent less (Dr. Earle). From the estimated measurements I should place its weight between 200 and 300 gm. The spleen was soft, pale red in color with multiple grayish white areas up to 25 mm. in diameter, and foci suggesting suppurative necrosis were seen on the cut surface.

Scattered mast myelocytes are seen in the blood in the lumen of large veins in both the liver and kidney, and in a postmortem extravasation of blood into the renal pelvis.

The other renal changes present are apparently independent of the leukemic process, consisting of severe diffuse degeneration of the cortical tubules, numerous hyaline casts in the tubules of the hyperemic pyramid, occasionally periglomerular, and rarely intraglomerular fibrosis and areas of cortical interstitial lymphocyte infiltration with endothelial proliferation in the included capillaries. The diagnosis of leukemia rests on the presence of typical myeloid infiltration of the liver and spleen, the marked splenomegaly, and the presence of myelocytes in the larger vessel lumina. Mobilization into the circulation does not appear to have been very great at the time of death, but some grade of myelocythemia was present.

In concluding I wish to acknowledge my indebtedness to Drs. Smith and Earle of the Division of Pharmacology for the material and gross notes.

### SUMMARY

A case of mast myelocyte leukemia in a cat is reported. Of some twelve reported or mentioned cases of leukemia in cats this is the fourth to be recorded as splenic or myelogenous and the first in cats in which basophilic myelocytes have been the predominating cell type. About nine other cases of mast myelocyte leukemia have been reported in man.

Anatomically this case presented an enormous enlargement of the spleen, enlargement of the liver and extensive mast myelocyte infiltration of the splenic pulp, mast myelocyte nodules in the liver and distention of liver capillaries by blood and large numbers of mast myelocytes. Numbers of mast myelocytes were seen in hepatic and renal veins. The blood, lymph glands and bone marrow were not examined.

The liver was about twice normal size, its surface mottled by slightly raised patches 2 to 10 mm. in diameter, white to deep red in color.

The kidney was pale, its surface rough and irregularly lobulated, the cortex was pale and waxy in appearance, the medulla red.

The heart and lungs appeared normal. There was some pallor but no other gross abnormality of the intestines, omentum or mesentery. No enlarged lymph glands were noticed.

Blocks of liver, spleen and kidney were fixed in 10 per cent formalin, in Ringer's fluid, buffered to pH 7.6 by Dr. W. R. Earle of the Division of Pharmacology, and forwarded to the writer for histological examination.

Unfortunately the rarity of the condition was not realized at the time and no blood smears were taken, nor were the long bones opened. Hence our histological study is limited to the liver, spleen, and kidney and to such evidence of leucocyte increase in the blood as can be derived from sections of larger blood-filled vessels.

Histologically the spleen shows small malpighian corpuscles, and its pulp is packed with mononuclear cells containing numerous small metachromatically basophilic granules. The nuclei are often obscured, but where plainly visible are large, vesicular in form, with scanty chromatin and one or more medium-sized nucleoli.

When stained with Weigert's iron chloride hematoxylin and safranin the granules are quite small, metachromatically orange-red and discrete, and the nuclei are regularly plainly shown, black in color, round or oval in form, vesicular with chromatin condensation about the periphery, and scanty, very fine chromatin granules in some nuclei, coarser, more plentiful chromatin blocks in others. In the former type large round gray-stained centrally placed nucleoli are regularly seen, while in the more pachychromatic nuclei nucleoli are less conspicuous or not distinguished from the nuclear chromatin. The leptochromatic type predominates in the spleen and in the liver nodules, being somewhat less preponderant in the liver sinusoids. An occasional mast metamyelocyte with elongate bean-shaped nucleus is seen in the latter location.

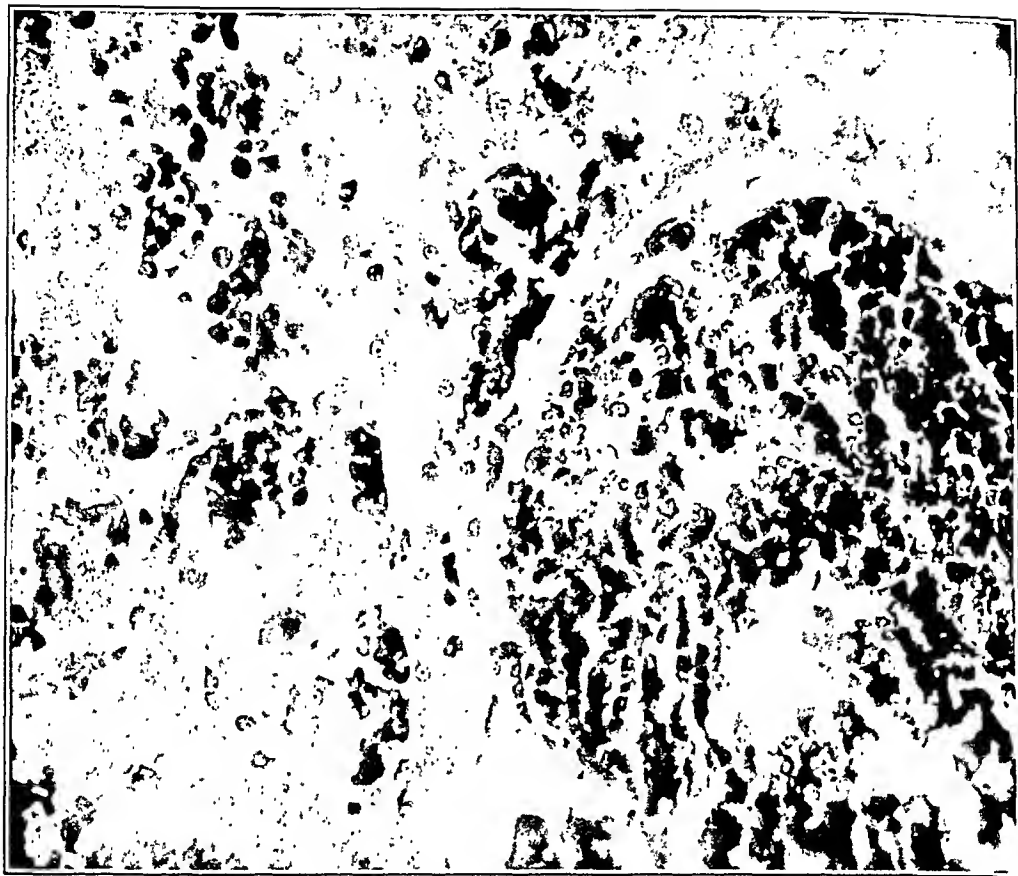
The liver sinusoids are dilated and blood-filled. In them are numerous single mononuclear basophil granulocytes, many large myelocyte clumps in the sinusoids, and nodules composed of mast myelocytes and few non-granular myeloblasts in the portal connective tissues.

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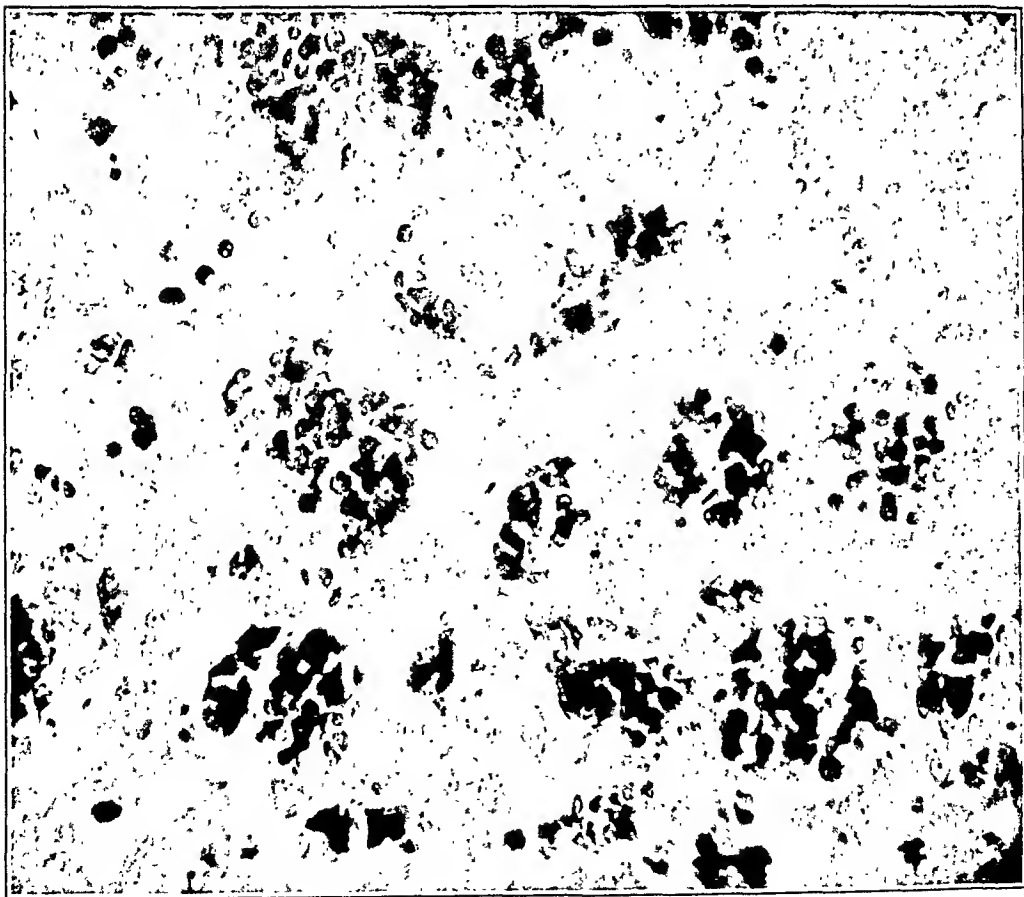
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1



## DESCRIPTION OF PLATES

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### PLATE 154

FIG. 1. Liver. Giemsa stain. E filter.  $\times 300$ .

FIG. 2. Liver, periportal nodule. Giemsa stain. E filter.  $\times 300$ .



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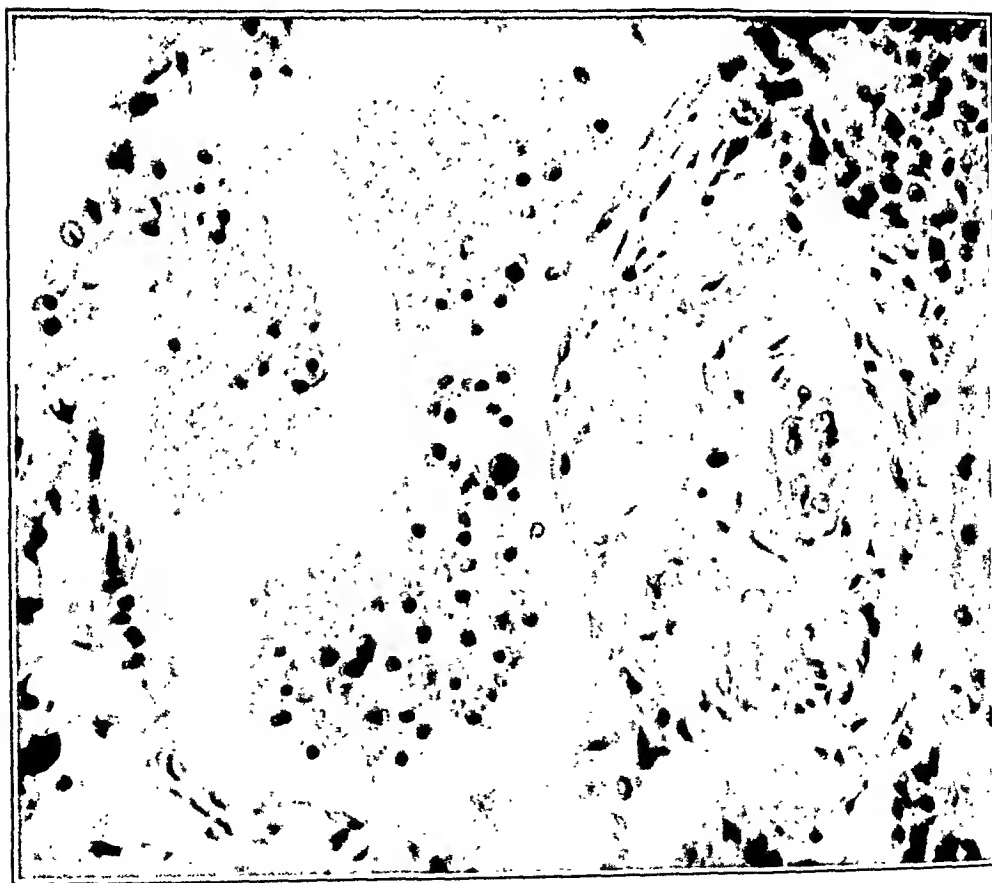


PLATE 155

FIG. 3. Liver, vein. Iron chloride hematoxylin and safranin stain. K-1 filter.  
× 400.

FIG. 4. Liver. Iron chloride hematoxylin and safranin stain. K-1 filter.  
× 1200.

matory infiltration. The epithelium was largely lacking. The inflammatory cells consisted of lymphocytes, rare polymorphonuclears and large numbers of plasma cells. Sections from the fundus, body and fovea were similar in appearance. Sections from the neck showed a more extensive process with foci of lymphocytes in the muscularis."

Fraenkel in collaboration with Schottmüller <sup>6</sup> in 1925 gave an excellent description of a case. They described a dense infiltration of the mucous membrane of a gall-bladder with lymphocytes and plasma cells. Focal clusters of similar cells were found in the muscularis and along its junction with the serosa. Organisms were demonstrable in the lumina of the crypts but not within the mucosa itself or the other layers of the wall. An excellent photomicrograph accompanying the article shows a typical lymph nodule with hyperplastic germinal center, though this is not mentioned in the text.

### DESCRIPTION OF CASES

Our own series of cases presented the following findings: In the seven cases of pure typhoidal infection the size of the gall-bladder was within normal limits, although two were moderately dilated. The wall was of normal thickness, thinned, or thickened up to 4 mm. in one instance. The mucosa was relatively thickened in proportion to the other layers; the rugae were prominent and in several cases the mucosal surface was definitely granular in appearance. In no instance was there fibrinous exudate or marked congestion of the serous surface. The content of the organ was in each instance pale, mucoïd and clear, or of only moderate turbidity. The color varied from colorless to a light yellowish green staining. Normal bile was not recovered. Stones were found in all but one case. They were usually small, dark in color and crumbled on slight pressure. In two instances they were as large as cherries and faceted. In only one instance was there calcification, and no cholesterol stones were found. Cultures of the bile showed *Bact. Typhosus* in pure culture. In one instance the organism was recovered from the center of a stone.

The microscopic picture varied within considerable limits but certain constant features could be recognized. In all of the cases an inflammatory infiltration was present, always most marked in the

PLATE 156

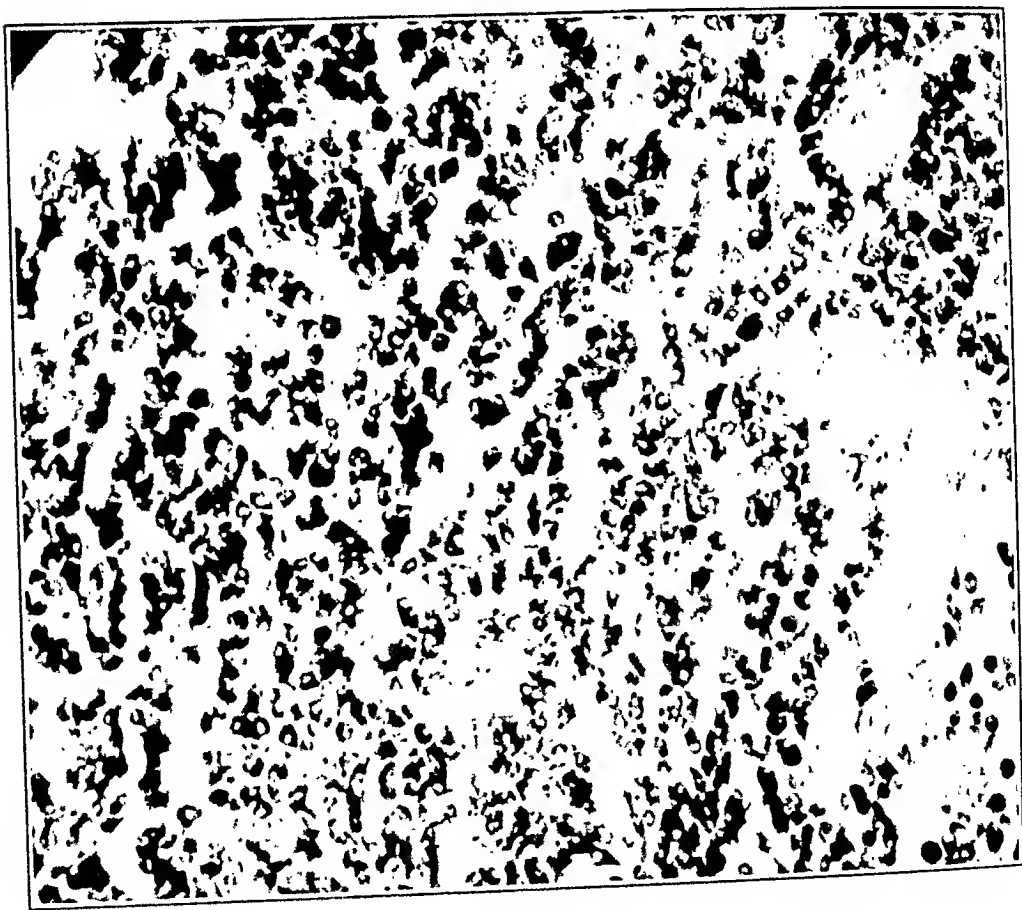
FIG. 5. Spleen. Giemsa stain. E filter.  $\times 300$ .

FIG. 6. Spleen. Iron chloride hematoxylin and safranin stain. K-1 filter.  
 $\times 1200$ .

6



5





# TUMORS OF THE ISLANDS OF LANGERHANS AND HYPOGLYCEMIA\*

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Pancreatic adenomas of island tissue are not as rare as the small number of reported cases would lead one to believe. In 1926 Warren<sup>1</sup> reported the occurrence of four such cases in the autopsy material which he studied, although he could find only sixteen reported in the literature up to that time. In Warren's report these tumors were characterized by resemblance to the islands in the arrangement and appearance of the cells, by an absence of mitotic figures, by the presence of a definite capsule and by compression of the adjacent tissue. He mentions in only one case the presence of granules characteristic of island cells. In this instance only beta granules were demonstrated. In the cases reviewed by Warren, diabetes occurred in one instance.

Gruber<sup>2</sup> calls attention to a case reported by Walz as a basal cell tumor of the pancreas. This tumor Gruber believes was an island cell adenoma. Two points about this case are of special interest: the patient was a diabetic and had also a carcinoma of the liver associated with cirrhosis.

Since Wilder's report<sup>3</sup> of an island cell carcinoma with hypoglycemia, one other case of adenoma without clinical symptoms of hypoglycemia has been reported by Lloyd.<sup>4</sup> This was a case with an adenoma of the hypophysis and tumor-like enlargements of the parathyroids and islands of Langerhans.

Previous to 1927 a number of authors discussed the possibility of certain carcinomas of the pancreas originating in island tissue, but this origin was never conclusively proved in any of the cases reported.

In 1927 Wilder published his remarkable case of carcinoma of the islands of Langerhans with hypoglycemia. Up to this time the literature contained no mention of the clinical correlation between

tumors of the island cells and hypoglycemia, although Harris<sup>5</sup> had previously called attention to cases with clinical symptoms of unexplained hypoglycemia. Wilder's patient was a physician who required hourly doses of glucose to prevent convulsions from spontaneous hypoglycemia. When ingestion of the necessary sugar was delayed, the blood sugar fell below 0.03 per cent. An autopsy revealed a carcinoma of the pancreas with metastases to the liver and lymph nodes. The cells of the pancreatic tumor bore a striking resemblance to the cells of the islands of Langerhans, and alcoholic extracts made from the metastatic tissue in the liver acted like insulin when injected into rabbits. The granules characteristic of island cells could not be demonstrated, perhaps because of improper fixation.

In 1928 Thalhimer and Murphy<sup>6</sup> reported a similar case in which symptoms continued with increasing severity for two and a half years, until the patient developed a state of almost constant semistupor, accompanied by frequent convulsions and marked hypoglycemia. Death occurred apparently from exhaustion. At autopsy the only significant pathological finding was a small tumor of the pancreas measuring 1.5 by 1 cm. Its microscopic appearance indicated that it originated from the cells of the islands of Langerhans, and it was called a carcinoma of low grade malignancy or an adenoma. The tissue was improperly fixed for staining cell granules, but Bensley was successful in demonstrating dust-like granules which stained very feebly in some of the tumor cells.

In 1929 Howland, Campbell, Maltby, and Robinson<sup>7</sup> reported another case which was successfully operated upon. In this patient dysinsulinism had existed for a period of six years, with attacks of coma and convulsions increasing in frequency. These attacks could be warded off by the administration of food and were found to be associated with low blood sugar levels. At operation a tumor measuring 1.5 cm. in diameter was found in the middle of the pancreas. This was removed. It was called a slowly growing carcinoma of island tissue. At the time of operation, search was made for possible metastases but none were found. The patient recovered and has since been entirely free from attacks. This was the only reported case successfully treated by removal of the tumor up to 1930.

In 1928 McClenahan and Norris<sup>8</sup> reported a case with periodic attacks of loss of memory and loss of consciousness with hypo-



glycemia. At autopsy there was found a large circumscribed adenoma of island tissue, together with hypertrophied islands in the remainder of the pancreas. This is the only one of these four cases associated with hypoglycemia in which the author made a definite diagnosis of benign tumor, but in two of the other cases the tumor, although called a carcinoma, was said to be very slowly growing. This is also the only one of the four cases in which the other islands in the pancreas were considered hypertrophied. The tissue, again, was improperly fixed for staining cell granules.

During the past year and a half we have observed at autopsy three cases of adenoma of the islands of Langerhans, one with a clinical history of hypoglycemia (Case 1), one with no clinical symptoms referable to the pancreas (Case 2), and one with mild diabetes (Case 5). In addition, we have seen surgical material from a case with hypoglycemia which was recently successfully operated upon at Barnes Hospital (Case 3),\* and material from an autopsy performed by Dr. Rabinovitch at the Missouri Baptist Hospital (Case 4).

### CASE REPORTS

**CASE 1.** (Autopsy No. 4120.) *Clinical History:* The patient, an electrician aged 40 years, was first admitted to the hospital February 3, 1925, with the chief complaint of dizziness and a dull aching headache in the back of his head. This had begun two years previously. He had had one attack of unconsciousness. The patient's past history showed the usual diseases of childhood. At 14 and 18 years of age he had chills and fever following gonococcal infections, and, at the age of 20 years, a chancre treated by cautery. The blood Wassermann taken at 28, 33, and 35 years of age was negative.

On physical examination the pupils were irregular, sluggish to light, but active to distant accommodation. The reflexes were all hyperactive. The blood Wassermann was negative. The spinal fluid showed a Wasserman + + + +, 26 lymphocytes, Pandy + + +, and a colloidal gold curve 2225532100. The patient entered the hospital for a lumbar puncture, which was done with no untoward symptoms.

In June 1926 the patient was again admitted for a lumbar puncture. The physical signs were essentially as above. There was no evidence of posterior column or pyramidal tract lesions. He had improved steadily under treatment with neosalvarsan and trypanseme and mercury injections. The blood Wassermann and Kahn reaction were negative as before. The spinal fluid Wassermann was now negative and the Pandy + +.

\* Another case of adenoma of the islands of Langerhans recently operated upon at the Peter Bent Brigham Hospital is referred to by Warren in a footnote (page 182) in his book, *The Pathology of Diabetes Mellitus*. Since the writing of this paper, a second case has been successfully operated upon at Barnes Hospital. This case is to be reported by Dr. Nathan Womack.

He was admitted a third time for a lumbar puncture, March, 1927. Since the second admission he had had ten to fifteen mild dizzy spells. On several occasions he had loss of consciousness, and on one occasion he had fallen. The physical findings were about the same as on the two previous admissions. At this time a brain tumor of the right frontal lobe was considered. The stereoscopic films of the skull were indeterminate and the evidence was considered insufficient to warrant an exploratory operation. The laboratory findings on this admission were: spinal fluid 2 lymphocytes; Wassermann negative; colloidal gold curve 12222100000; urine negative, phenolsulphonephthalein 21 per cent in two hours.

In October, 1927 the patient was admitted for the fourth time as an emergency, having recently become stuporous and irrational. An intracranial lesion seemed possible. At times he would spontaneously open his eyes, look at one with a silly grin, turn over on his side, and fold his arms about his head. At this time while stuporous, he had a bilateral ankle clonus but no abnormal toe signs. Later, while he was still in a stupor, he had both a bilateral Babinski and an ankle clonus, but the next day, October 4, when the patient was no longer stuporous but rather alert, the ankle clonus had disappeared and the Babinski remained positive only on the right side. October 5 the Babinski on the right side was considered doubtful. At this time positive signs of a brain tumor were again considered insufficient for operation. The spinal fluid and the blood Wassermann were still negative. He improved without specific treatment and was discharged October 12, 1927.

October 25th, 1928 he reentered for a lumbar puncture, his fifth admission. There had been practically no specific treatment for the past year, but he had improved somewhat. The patient thought his speech was slower than formerly and that his mental ability had decreased. He appeared emotionally unstable. The physical signs were essentially as on the first admission. There were no abnormal toe signs and no ankle clonus. A lumbar puncture showed a fluid with a negative Kahn and Wassermann reaction, a colloidal gold curve of 444552000, and 4 lymphocytes. The phenolsulphonephthalein was 35 per cent in two hours.

The patient's final and sixth entry was on June 7, 1930, when he was sent into the hospital as an emergency case. He was unconscious and practically in shock, and had been brought from Topeka, Kansas in this condition. The history obtained from his wife disclosed that he had had occasional spells of unconsciousness for the past three months. These attacks had begun with weakness of legs, numbness of hands and mental confusion. Eventually he would fall and remain unconscious from one to twelve hours. During these attacks he had shown a marked emotional disturbance, laughing foolishly, making peculiar grimaces, kicking his feet in the air and jumping in and out of bed. The present period of unconsciousness had begun two days before entry.

On admission he was comatose. The skin was cold and clammy. There was marked dehydration and there were coarse râles throughout the chest. The pupils were unequal and sluggish. There was a left facial weakness and a bilateral Babinski. The laboratory findings were: red blood count 6,000,000; hemoglobin 90 per cent; white blood count 19,700; blood Wassermann and Kahn negative; spinal fluid Wassermann and Kahn negative; colloidal gold curve 00122100000; Pandey ++; one lymphocyte. Urine negative; blood non-protein nitrogen normal. Blood sugar 58 mg. per 100 cc. of blood. His temperature rose rapidly to 39 C and signs of pneumonia developed in the left

lower lobe. A blood culture was positive for *Streptococcus viridans*. The temperature rose rapidly on June 10 and the patient died early the following morning. The temperature just before death was 41.8 C.

In brief, the patient, a white male 40 years of age with a definite history of syphilis and having on one occasion a four plus spinal fluid Wassermann, complained of peculiar attacks of dizziness and unconsciousness over a period of five years. At first these attacks appeared infrequently. Later they increased in frequency and lasted from one to twelve hours. Signs of an anatomical lesion in the central nervous system were transient and variable. On the final admission, the patient had been unconscious for two days and remained unconscious for four days in the hospital, when he died from pneumonia.

The laboratory report of the blood sugar determination was not returned until after the autopsy report was made.

### *Anatomical Findings in Brief*

The body is that of a well nourished male adult of 40 years, weighing 75 kilos and measuring 175 cm. in length. Rigor mortis has not developed. There is no adema. On the right ankle and foot there is a skin lesion which resembles impetigo contagiosa. Pupils are equal and regular.

The heart, aorta, spleen, stomach and intestines are normal. With the exception of the pancreas, the glands of internal secretion are normal.

The lungs are covered with a smooth and glistening pleura. The left lower lobe feels non-crepitant. On section the cut surface of this lobe shows areas which are red and consolidated. These areas have a patchy distribution throughout the lobe. Considerable fluid oozes from both lungs. The bronchi and trachea show no changes.

The liver weighs 1420 gm. and measures 20 by 10 by 7.5 cm. Its capsular surface is rough and uneven, due to projections of small irregular nodules of liver tissue above intervening connective tissue scars. On cut section there is a marked increase in connective tissue separating irregular nodules of liver tissue.

The pancreas weighs 80 gm. and measures 19 by 4 by 2 cm. It appears perfectly normal, perhaps a little smaller than usual. No nodules or irregular firm areas can be felt. On section there is seen

in the center of the head a round circumscribed nodule 1 cm. in diameter. This nodule is a little more vascular than the surrounding tissue. It is not divided into the normal pancreatic lobules, but seems almost homogeneous in structure. Its color is gray, in contrast to the yellowish gray color of the rest of the organ.

No abnormalities are seen when the skull is opened. The brain shows normal convolutions and the meninges are everywhere thin and transparent. The blood vessels, including those at the base of the brain and in the meninges, seem entirely normal. When the brain is sectioned, no gross abnormalities are found.

### *Microscopic Examination*

*Lung:* There is an extensive polymorphonuclear exudate throughout all the sections examined.

*Kidney:* The tubular epithelium shows slight cloudy swelling.

*Liver:* There is an abundance of connective tissue about the portal spaces. This extends out into the liver lobules dividing the liver tissue irregularly. There are many mononuclear cells in this connective tissue and new-formed bile ducts are frequent. There has been little proliferation of liver cells.

*Brain:* Several sections show no changes in the brain itself. The meninges are quite normal. The small blood vessels in the brain substance and in the meninges are normal. There is no cellular infiltration about vessels either in the brain or in the meninges.

With the exception of the pancreas other organs shows no microscopic abnormalities.

*Pancreas:* The pancreas, at a distance from the tumor, shows a slight increase in interlobular connective tissue. In another section the tumor nodule is irregular in outline, but everywhere is sharply demarcated from the surrounding pancreatic tissue. Throughout most of its circumference it is surrounded by a fairly thick connective tissue capsule, but in a few places it is separated from the normal pancreatic tissue only by a very thin strand of connective tissue, scarcely more than the capsule about a normal island. The tissue about the tumor nodule is compressed here and there, and there is more scarring in this region than elsewhere in the pancreas. Normal islands are seen near the tumor, but never continuous with it. In the nodule there are strands and masses of cells divided by an inter-

lacing connective tissue stroma that is almost acellular and has a homogeneous hyaline character. Capillaries are fairly numerous in this connective tissue. The tumor cells often border the capillaries. The peculiar connective tissue stroma suggests a resemblance to the hyaline tissue seen in the so-called hyaline islands. The tumor cells all stain alike with hematoxylin and eosin. They have an abundance of cytoplasm which stains with eosin like the cytoplasm of island cells, in contrast to the more basophilic cytoplasm of acinar cells. The nuclei are round or slightly oval. They are much like those of normal island cells.

In a section of pancreas fixed immediately at autopsy in Zenker-formol the cells of the normal islands have a normal content of beta granules when stained with Bensley's neutral gentian or Bowie's stain. Alpha granules do not stain. Material from the tumor was not fixed until later, so that the granular content of the cells is poorly preserved, but one can find individual cells in the tumor which show a few of the characteristic small blue beta granules which stain with Bensley's neutral gentian or Bowie's stain. This seems to prove beyond doubt that the tumor is derived from island tissue and that at least a part of the cells contain beta granules. The material is such that a detailed study is not possible.

*Anatomical Diagnoses:* Pancreatic adenoma of island tissue; cirrhosis of liver; bronchopneumonia; impetigo contagiosa of right foot.

### *Comment*

The periodic attacks of dizziness, loss of consciousness and coma, with the peculiar emotional reactions which this patient experienced over the period of observation, are explained by a functioning tumor of the islands of Langerhans. There is a record of only one blood sugar determination, but this one determination showed a definite hypoglycemia. This case is of special clinical interest because of the obscuring influence of a suspected central nervous system lesion. The laboratory evidence of syphilis of the central nervous system at the beginning of this man's history colored the interpretation of the clinical symptoms throughout the course of his illness. Yet these clinical symptoms were so atypical of syphilis that a diagnosis of brain tumor involving the frontal lobe was also seriously considered. When one considers the descriptions of the

mucous membrane and often sharply limited to it. Usually a few scattered lymphocytes and an occasional lymph nodule were found on the outer border of the muscularis but the process was always very slight in extent compared with that in the mucous membrane. The latter was considerably thickened as the result of a diffuse infiltration of lymphocytes and plasma cells, the former predominating. Just beneath the epithelium the capillaries were dilated and contained numerous polymorphonuclear cells. A few of these cells were found extravascularly in the same region and considerable numbers lay between the epithelial cells, apparently in transit to the lumen of the organ. They were rarely found deeper in the mucosa in the zone of lymphocytic infiltration, although small numbers of eosinophiles were present here in one case.

In addition to the diffuse lymphocytic infiltration, dense focal clusters of lymphocytes in typical lymph nodule formation with hyperplastic germinal centers of immature cells were found in every instance. They were numerous enough to average three to four in each microscopic section and were apparently present in approximately equal numbers in sections from all portions of the wall from neck to fundus.

Microorganisms, though numerous in the bile, could not be demonstrated in any of the layers of the wall. Cholesterosis was not present in any case.

### DISCUSSION

Before attempting to interpret the figures in this tabulation the possible sources of error must be considered. On the one hand a false diagnosis of typhoid may have been made, on the other hand three factors would lead to missing positive cases. By all odds the most important of these is inadequacy of the past history either from neglect or because of language difficulties in the many foreign-born patients of a general clinic. Two other definite factors also may play a rôle. "Walking" or abortive cases of typhoid are not very rare and primary typhoidal cholecystitis has been reported by numerous authors. Since the positive diagnosis of typhoid fever is reasonably accurate it seems probable that our figures show too few, rather than too many past histories of typhoid.

Thirty-two, or 8 per cent of our cases gave a history of typhoid fever, and of these, eight or 25 per cent showed the characteristic

inquiry was made into the man's history taken in the hospital where he was under observation for six weeks before his death. Not the slightest clinical evidence of hypoglycemia was recorded. Unfortunately no blood sugar determination had been made.

This adenoma was located near the middle of the body of the pancreas. It was of approximately the same size as that in Case 1, in which hypoglycemia was demonstrated. The tumor was recognized grossly, when the pancreas was sectioned, by its gray color which contrasted with the yellowish gray color of the surrounding pancreatic tissue, and by its lack of normal pancreatic lobules. Grossly it showed no evidence of increased vascularity.

Microscopically, with a hematoxylin and eosin stain, one finds a tumor nodule surrounded by a rather thick capsule of connective tissue. This capsule completely surrounds the nodule, but in places there are islands of tumor tissue in the capsule in very close contact with the normal pancreatic tissue. In other places normal islands and a few acini are seen within the capsule and bands of connective tissue extend into the tumor from the capsule. The pancreatic tissue outside this capsule shows a moderate amount of interacinar scarring. A few acini are distended and contain a little colloid-like material. The tumor cells are arranged in interlacing bands and small clusters. In some areas they seem less well preserved and have no definite arrangement but form a group of isolated cells. In such places the cells stain a little more intensely with eosin than in the rest of the tumor. Between the columns of cells there is a conspicuous homogeneous connective tissue stroma, much like that described in Case 1, but even more conspicuous. The capillary channels are sometimes quite wide and filled with blood. The individual cells in this tumor resemble island cells. They are of about the same size. Their cytoplasm is eosinophilic rather than basophilic like that of the acinar cells, but the nuclei are on the whole somewhat smaller than those of the normal island cells and the chromatin material is denser. The islands near the tumor and in another section taken at a distance from the tumor are not abnormal in size.

With Bowie's stain, following Zenker-formol fixation, the cells in the normal islands show a normal content of beta granules, but alpha granules are not recognized. In the tumor, however, many of the cells contain small indistinct granules which take a reddish stain. In other cells a few normal beta granules occur mixed with the red-

attacks, comparing them with the descriptions in previous reports, they seem quite characteristic of hypoglycemic manifestations. In all of the reported cases the attacks have been similar. Besides the actual coma and convulsions there are certain characteristic symptoms which are mentioned repeatedly: the mental daze and loss of memory, peculiar grimaces, aimless movements of arms and legs, and the display of an amiable or even jocular mood when aroused from stupor. Profuse perspiration at the termination of attacks is common.

The small size of this tumor, together with the fact that it did not contrast sharply with the normal pancreatic tissue shows the possibility of overlooking such a tumor even at autopsy.

In this connection a case such as that reported by Finney and Finney<sup>9</sup> is of interest. Their patient, a woman aged 53 years, with a psychopathic background, had hysterical attacks with stupor and unconsciousness associated with a low blood sugar. An operation was performed. As no tumor was discovered the operators removed a large part (22.5 gm.) of the normal pancreas. The patient recovered from the operation. The blood sugar, although somewhat higher than previously, continued abnormally low. The symptomatic attacks were believed to be lessened in frequency and severity, but were still of a character to incapacitate the patient. It seems not unlikely that a tumor of small size remained undiscovered in the part of the pancreas not removed.

Likewise two cases operated upon at the Mayo Clinic and one operated upon by Holman, all referred to by Allan, *et al.*,<sup>10</sup> have shown similar courses with slight but unsatisfactory improvement. In each of these three cases no tumor was found at operation and a part of the normal pancreas was removed.

That such cases represent hypertrophy or hyperfunction of normal islands, or even some abnormality outside the pancreas, rather than an undiscovered tumor is, of course, possible. But, realizing how indistinct an adenoma may be even at autopsy, one is left in doubt as to the absence of a tumor in such cases.

Two of the other adenomas which we have seen help to explain the nature of these tumors.

CASE 2. (Autopsy No. 3950.) In this instance the adenoma was an incidental finding at autopsy in a man dying from a primary liver cell carcinoma. After the discovery of the pancreatic tumor a careful



resembles the surgical material in Case 3. The history of the patient is vague. He was a man 50 years of age, operated upon for intestinal obstruction because of symptoms of vomiting and abdominal pain. No obstruction was found but a gall-bladder filled with stones. He died following the operation. No satisfactory cause for the clinical symptoms or for his death was found at autopsy. Inquiring into the history, after the finding of the small pancreatic adenoma, the interesting information was gained that his family considered the man mentally defective, because of periods of loss of memory.

CASE 5. This case differs from the other four both in the clinical history and in the character of the tumor. This patient was a colored woman of 48 years, who entered the hospital during the course of an acute lobar pneumonia. Several days after the crisis of the disease she died suddenly of a pulmonary embolus which originated in the femoral vein. Her past history gave no record of symptoms referable to a pancreatic lesion, but during the course of this acute infection she behaved as a total diabetic.

At autopsy, the anatomical findings were briefly as follows: resolving lobar pneumonia; extremely large multiple myomata uteri; cardiac hypertrophy; chronic passive congestion of viscera; thrombosis of left iliac and femoral veins; pulmonary embolus; adenoma of islands of Langerhans; cavernous hemangioma of liver.

Only the pancreatic lesions are of interest to us here. Grossly, the pancreas is slightly smaller than usual, weighing only 80 gm. A well encapsulated nodule, measuring 1 cm. in diameter, is seen near the center of the body of the pancreas on its anterior surface. This nodule is paler than the rest of the organ and does not show the regular lobulations of the normal tissue. It is not more vascular than the surrounding pancreas.

In a microscopic section which includes the nodule, the pancreatic tissue shows a slight interacinar and interlobular scarring. An occasional acinus shows some distention of its central lumen. Most of these are at some distance from the edge of the nodule. The ducts show no evidence of obstruction. The islands of Langerhans are of average size and number. With a hematoxylin and eosin stain they show nothing abnormal. About the nodule there is some compression of the pancreatic lobules and more scarring than elsewhere. The nodule is composed of lobules of tissue separated from the normal

dish granules, while still other cells are filled with beta granules like those of the beta cells of normal islands. The beta granules are frequently arranged at one pole of the cell. With no other material to help in the interpretation of this case, we might have come to the conclusion that this tumor was composed only in part of beta cells with a large number of cells which were probably alpha cells. A similar appearance is described by Robinson in the case reported by Howland, Campbell, Maltby and Robinson and is interpreted by Robinson as indicating the presence of both alpha and beta cells in the tumor, sometimes both alpha and beta granules occurring in the same cells. Fortunately very fresh surgical material from a patient (Case 3) operated upon by Dr. A. O. Fisher and reported by Carr, *et al.*,<sup>11</sup> was studied here by Bensley, and throws light on the character of these cells.

CASE 3. This patient, a young boy, had shown characteristic symptoms of hypoglycemia for at least one and a half years. The tumor removed from the body of the pancreas measured 2 by 1.5 cm. When fixed in Zenker-formol this surgical material stained much like the material just described (Case 2, Autopsy 3950), but fewer tumor cells were seen containing normal beta granules. A few normal islands in the attached pancreatic tissue showed a normal content of beta cells, but Bensley also fixed material in acetic-osmic-bichromate mixture and stained it with acid fuchsin-picric acid. This method which stains alpha granules well, failed to demonstrate them in the tumor cells. Fresh tissue treated with neutral red showed many cells of the tumor packed with granules taking up the neutral red dye, a characteristic of beta as well as alpha cells, while other cells contained small granules which did not seem to take up the dye. This fact, he believed, showed that the unusual cells in the tumor were not alpha cells but modified beta cells, a condition which one might expect in tumor tissue.

CASE 4. This case adds nothing more to our knowledge of the character of these tumors but helps to emphasize their frequency. The tumor was also an incidental finding at autopsy and the material was not ideally fixed, although it was possible to stain beta granules in some cells of the tumor and in some normal island cells with Bowie's stain. It measured only 6 mm. in diameter. Microscopically, with hematoxylin and eosin, the tumor although small closely

This tumor is unlike the others discussed in this paper, in that it gives neither clinical nor anatomical evidence of functional activity. In addition, this is the only case in which a definite although mild diabetes was present.

### DISCUSSION

In all our cases there might be a difference of opinion as to whether they should be called adenomas or slowly growing carcinomas. We are inclined to consider them all as adenomas. The more important conclusion seems to be that they are definite tumors and cannot be considered merely as hypertrophied islands.

Only two island tumors associated with hypoglycemia in which the tumor material has been properly fixed for complete cytological studies (the one described by Robinson and the one studied here by Bensley) have shown atypical cells, probably atypical beta cells, although not so interpreted by Robinson. All the cases associated with hypoglycemia, except that reported by McClenahan and Norris, have shown islands of normal size in the remainder of the pancreas. In their case the islands were considered hypertrophied. In those instances (Cases 1, 2, and 3 in this report) in which material has been stained to demonstrate granules in the island cells outside the tumor nodule, these cells have shown a normal granular content. The activity of the normal islands, as determined by this cytological method, does not seem to have been depressed by the excess of insulin produced in the tumor. Nor is there evidence in the cases with hypoglycemia, except perhaps in the case of McClenahan and Norris with hypertrophied islands, to show that the normal islands have been abnormally stimulated. The recovery following removal of the tumor in two cases also shows that there was no overstimulation of the normal islands.

This apparent lack of coördination between the tumor and normal islands is probably linked with functional differences between tumor and normal cells. These cells may therefore not respond to the normal mechanism of insulin control. However, the failure to find clinical symptoms in our Case 2 is not explained by such a theory, for there the tumor also contained many of these abnormal cells and was about as large as the tumor in Case 1 which gave symptoms. The lack of blood sugar determinations leaves us in doubt about this case.

pancreas and often from each other by strands of connective tissue. Pancreatic tissue — acini, ducts and islands — are found in the connective tissue capsule, but are distinctly separated from the tumor tissue. However, at times this separation is by a very thin strand of connective tissue. At one side of the nodule there are two large ducts. The wall of one of these is encroached upon by the tumor, so that a mass of the tumor tissue bulges into the lumen of the duct and is covered only by a flattened layer of duct epithelium. This apparent invasion of the duct suggests a malignant growth, but elsewhere the arrangement of cells is so regular and the character of the cells so uniform that one hesitates to speak of this as a carcinoma. The arrangement of cells within the lobules is that of island tissue. There are interlacing strands and circles of uniform cuboidal or columnar cells bordering capillaries. No mitoses are seen. The nuclei are slightly smaller than those of the normal island cells. They are all quite similar in size and in staining qualities.

Although their arrangement suggests island tissue, the cells are more regular in size and shape and are more distinctly columnar than island cells. This character, but not the arrangement of cells, suggests the possibility of origin from duct cells, but when stained with mucicarmine the cells do not contain the drops of mucin seen in the duct epithelium.

With Bowie's stain after Zenker-formol and after Zenker-acetic fixation, no granules taking the specific stain are seen in the cells of the tumor, although the cells in the islands show a normal content of stainable granules, and the acinar cells contain zymogen granules. With a modified Mallory connective tissue stain, using aniline acid fuchsin instead of acid fuchsin, no red granules are seen in the tumor cells, although the blue-stained cytoplasm seems distinctly granular. With the fixations used, the possibility of the presence of alpha granules is not entirely eliminated. Certainly no normal beta granules are present and the existence of abnormal beta granules such as those seen in some of the other adenomas is not demonstrated. Only its structure leads one to believe that this tumor is derived from island tissue. We do not believe that it can be interpreted as a hypertrophic island, for Bensley has called attention to a quite different appearance in such islands. In hypertrophied islands produced by experimental obstruction of comparatively long duration he observed that the cells were always packed with normal granules.

TABLE I  
*Results of Glucose Tolerance Tests*

## I

*Wilder's Case*

120 GM. GLUCOSE		100 GM. GLUCOSE 15 GM. $\frac{1}{2}$ Hr. BEFORE ZERO Hr.	
Time	Blood Sugar	Time	Blood Sugar
0.....	68 mg. per 100 cc.	0.....	85 mg. per 100 cc.
$\frac{1}{2}$ hr.....	242 mg.	1 hr.....	..
1 hr.....	283 mg.	2 hr.....	214 mg.
2 hr.....	223 mg.	2 $\frac{1}{2}$ hr.....	..
3 hr.....	70 mg.	3 hr.....	31 mg.

## II

*Finney's Case*

Time	AMT. GLUCOSE NOT RECORDED	Blood Sugar
$\frac{1}{2}$ hr.....		192 mg. per 100 cc.
1 $\frac{1}{2}$ hr.....		135 mg.
2 $\frac{1}{2}$ hr.....		112 mg.
Fasting sugar 52 mg. per 100 cc.		

## III

*Hartmann's Case of Hypoglycemia.<sup>12</sup> Tumor of Islands Suspected  
but not Proved*

120 GM. GLUCOSE		200 GM. GLUCOSE	
Time	Blood Sugar	Time	Blood Sugar
$\frac{1}{2}$ hr.....	135 mg. per 100 cc.	0.....	74 mg. per 100 cc.
1 hr.....	166 mg.	2 hr.....	242 mg.
2 hr.....	216 mg.	3 hr.....	140 mg.
3 hr.....	100 mg.	4 hr.....	66 mg.
Fasting sugar 53 mg. per 100 cc.			

## IV

*Robinson's Case*

Time	100 GM. GLUCOSE	Blood Sugar
1 hr.....		250 mg. per 100 cc.
2 hr.....		260 mg.
3 hr.....		240 mg.

## V

*Case III of this Report*

155 GM. GLUCOSE		155 GM. GLUCOSE	
Time	Blood Sugar	Time	Blood Sugar
$\frac{1}{2}$ hr.....	134 mg. per 100 cc.	$\frac{1}{2}$ hr.....	172 mg. per 100 cc.
1 hr.....	132 mg.	1 hr.....	183 mg.
2 hr.....	135 mg.	2 hr.....	123 mg.
3 hr.....	102 mg.	3 hr.....	60 mg.
Fasting sugar 81 mg. per 100 cc. (first admission)		Fasting sugar 72 mg. per 100 cc. (third admission)	

In Case 3 the sugar tolerance curve, in addition to the anatomical study, gave no evidence of change in the normal islands. This is not true of all of the cases, however. The sugar tolerance curves in some of the reported cases of hypoglycemia associated with pancreatic tumors have shown what can be interpreted as a low sugar tolerance, that is, the attainment of an unusual high blood sugar level and a slow return to normal. This suggests some depression of the activity of the normal islands, but corresponding anatomical studies of the islands in properly fixed material is lacking in those cases which showed this peculiar type of sugar tolerance curve.

The occurrence of mild diabetes in one of our cases with a non-functioning tumor and, also, in two cases of island adenoma reported in the literature, suggests another possible explanation of this low sugar tolerance in the cases with a functioning tumor. Perhaps in both instances the low sugar tolerance is dependent upon some unexplained developmental defect of the pancreatic islands which also accounts for the growth of the adenomas. In those cases with a similar adenoma without definite diabetes, or without a low sugar tolerance, perhaps the same sort of developmental defect has manifested itself only in the occurrence of the adenoma.

Cecil<sup>13</sup> described similar structures, usually in diabetics, which he believed were hypertrophied islands. Although it may, at times, be difficult to distinguish between adenomatous proliferation and hypertrophy, the following facts argue for the occurrence of true island adenomas. It is known that the normal pancreas is capable of producing many times as much insulin as is required, but it is regulated to the needs of the body. The adenomas are not controlled by the normal mechanism governing insulin liberation and may produce hypoglycemia in spite of their small size. The stimulus that produces their increased growth and function does not affect the normal islands in a corresponding manner. The cells in these tumors are not like the normal island cells. In some of the tumors the cells contain abnormal secretory granules. In others, they have no secretory granules taking specific stains, although the island cells have such granules in apparently normal numbers.

Attention should be called to a possible relation between tumors of the liver and island cell adenomas. One of the adenomas reported here was in an individual dying of a primary liver cell carcinoma. In the case of Walz (discussed by Gruber) a primary liver cell carci-

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noma, as well as the pancreatic adenoma was present. Borst (quoted by Ewing<sup>14</sup>) mentions the occurrence of localized hyperplasias of the pancreas and of the liver in the same individual. In light of these observations two reported cases<sup>15,16</sup> of liver cell carcinoma associated with severe hypoglycemia without other gross evidence of hepatic dysfunction are of interest. In these cases no pancreatic tumor was discovered.

### CONCLUSIONS

From the cases reported in this paper and those found in the literature one may draw the following conclusions:

1. Pancreatic tumors producing hypoglycemia are composed largely of abnormal beta cells.
2. In those cases with hypoglycemia due to a pancreatic tumor of island tissue the normal island cells are not overstimulated.
3. From anatomical studies in these cases there is no evidence that the activity of the normal island cells is depressed, although clinical studies of the sugar tolerance suggest this in some cases.
4. Adenomas resembling islands of Langerhans in their cellular arrangement, which give neither clinical nor anatomical evidence of functional activity, may occur.
5. Adenomas of the islands of Langerhans are not rare; even those with clinical symptoms are surprisingly frequent.

NOTE: We wish to thank Dr. R. R. Bensley for his helpful advice and criticism.

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histological picture found in our known typhoid carriers. In contrast, of the three hundred and sixty-eight cases with no history of typhoid only eighteen, or 4.9 per cent showed this type of pathology. The five-fold higher percentage seems certainly significant. Assuming that with more accurate histories 50 per cent more positive histories could be obtained, the difference between the two series would

TABLE I

*Correlation between Histological Findings and Clinical History in 400  
Consecutive Cases of Chronic Cholecystitis*

	Number	Per cent
Cases with no history of typhoid .....	368	92.0
Cases with positive history of typhoid .....	32	8.0
Cases showing positive "typhoid" histology .....	26	6.5
Cases showing negative "typhoid" histology .....	358	89.5
Cases showing doubtful "typhoid" histology .....	16	4.0
Cases with negative history showing positive histology .....	18	4.9
Cases with negative history showing negative histology .....	336	91.3
Cases with negative history showing doubtful histology .....	14	3.8
Cases with positive history showing positive histology .....	8	25.0
Cases with positive history showing negative histology .....	22	68.8
Cases with positive history showing doubtful histology .....	2	6.2

still be between three- and four-fold. The fact that 68.8 per cent of our cases with positive histories showed no histological evidence of typhoid infection is of course not surprising since only a small percentage of cases of typhoid pass on into the carrier stage.

The eighteen cases with "positive" histology but no history of typhoid are certainly too many to be explained away on the basis of inaccurate histories or unrecognized attacks and we feel that an identical pathological picture can unquestionably be produced by other organisms. The findings of Wilkie <sup>7</sup> and others are interesting in this connection. He found that in the majority of cases of chronic cholecystitis the bile was sterile, while cultures from the outer layers of the bladder wall, or especially the cystic node, were usually positive. *B. typhosus* and a few related organisms grow comparatively readily in bile and it seems possible that the histological picture de-

## DESCRIPTION OF PLATES

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### PLATE 157

- FIG. 1. Longitudinal section showing adenoma of island tissue in the middle of the head of the pancreas (Case 1).  $\times 0.8$ .
- FIG. 2. Cross-section of pancreas showing adenoma of island tissue (Case 2).  $\times 0.8$ .
- FIG. 3. Low power photomicrograph of portion of the adenoma of island tissue shown in Fig. 1 (Case 1).
- FIG. 4. Low power photomicrograph of adenoma of island tissue shown in Fig. 2 (Case 2).

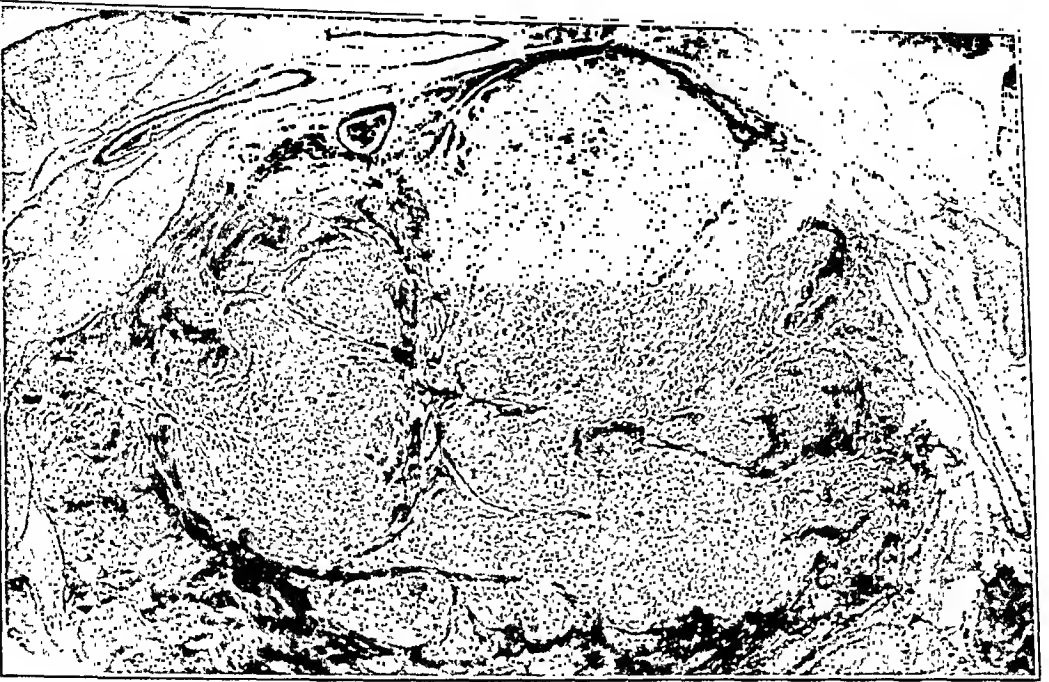
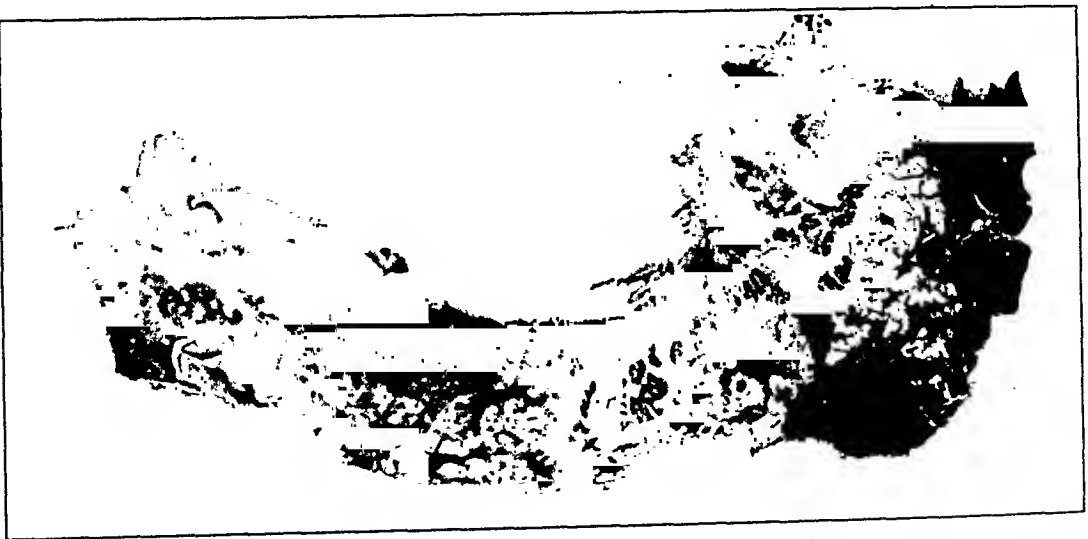
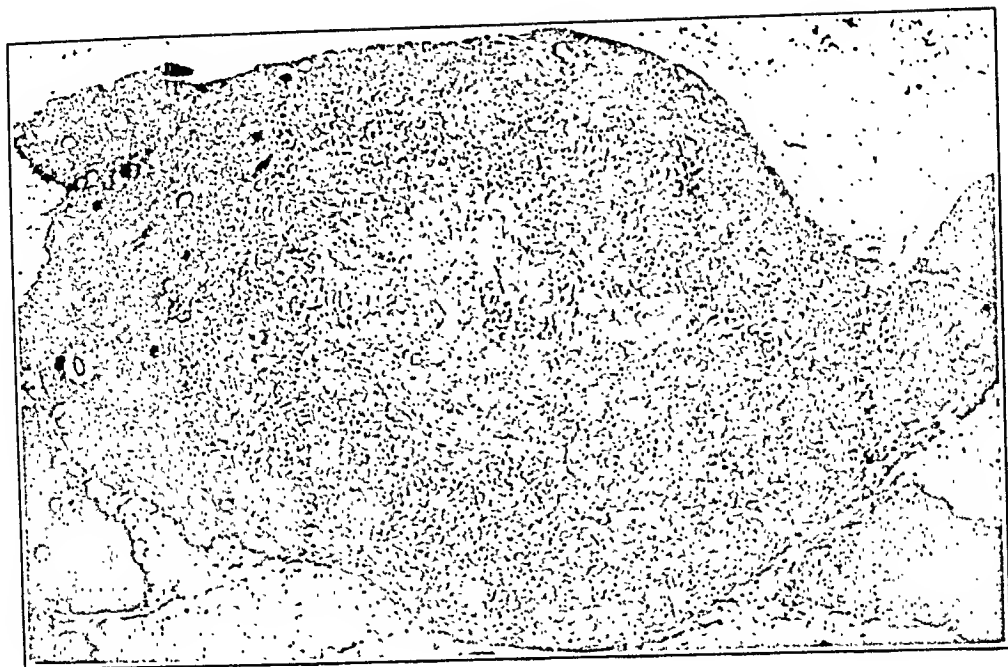
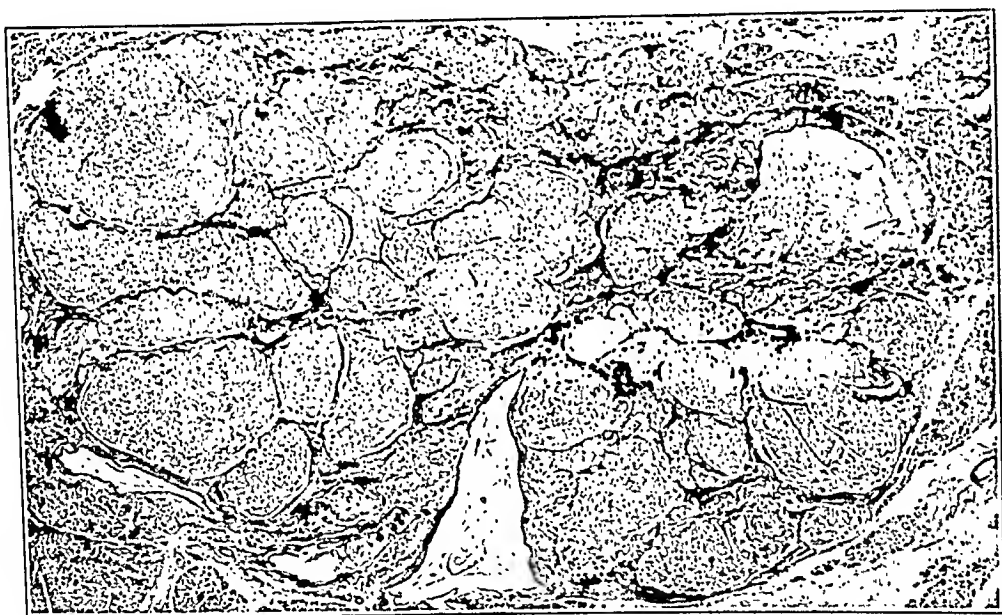


PLATE 158

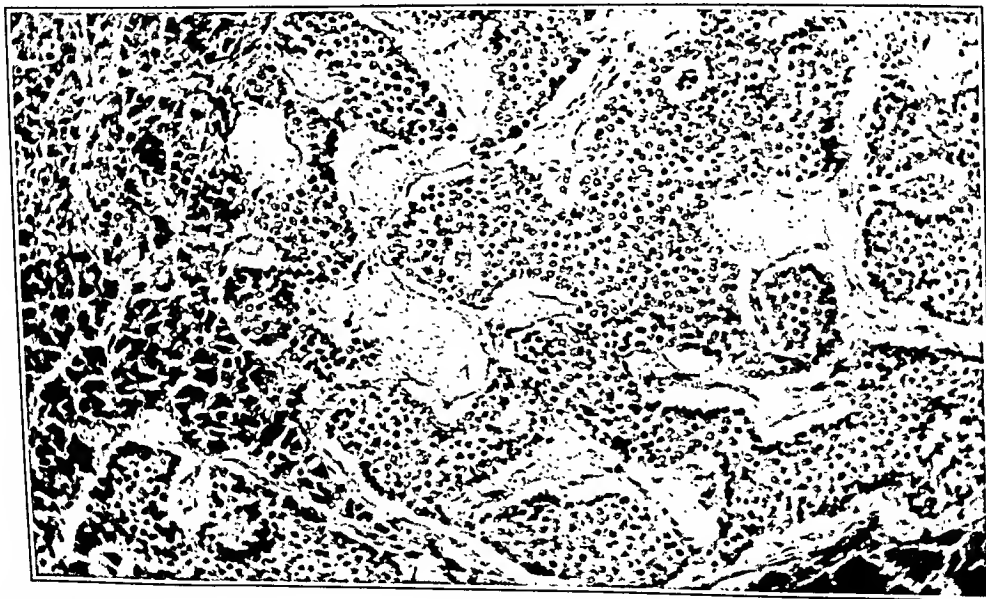
- FIG. 5. Low power photomicrograph of adenoma of island tissue from Case 4.
- FIG. 6. Low power photomicrograph of adenoma of island tissue from Case 5.
- FIG. 7. Higher magnification of adenoma shown in Figs. 1 and 3 (Case 1). The hyaline connective tissue between groups of tumor cells is shown. Compressed pancreatic tissue extends between two lobules of the tumor.



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6



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PLATE 159

- FIG. 8. Higher magnification of adenoma shown in Figs. 2 and 4 (Case 2). The hyaline connective tissue is seen between strands of tumor cells.
- FIG. 9. High power photomicrograph of adenoma of island tissue removed at operation (Case 3). The hyaline connective tissue stroma is conspicuous.
- FIG. 10. Higher magnification of adenoma shown in Fig. 5 (Case 4). The connective tissue stroma is again conspicuous.
- FIG. 11. Higher magnification of adenoma seen in Fig. 6 (Case 5). The capsule about the tumor is shown. The hyaline connective tissue in the tumor is prominent.

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\* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at Cleveland, Ohio, April 2 and 3, 1931.

scribed is typical not of any specific organism but of primary infection of the bile rather than of the gall-bladder wall. This hypothesis would fit the histological picture well, since the lesion in no way resembles the specific histology of the acute stage of typhoid.

### SUMMARY

The gross and histological appearance of the gall-bladders of seven typhoid carriers are described. A common histological picture was found. A review of four hundred routine slides of chronic cholecystitis showed a similar lesion in 6.5 per cent of the cases. A review of the clinical histories showed that only 4.9 per cent of the cases with negative histories showed this picture as against 25 per cent of cases with positive histories. It is felt that the lesion described is characteristic of chronic typhoidal cholecystitis but not pathognomonic of it. It is suggested that it is not specific of any organism but represents a reaction to persistent infection of the bile in contrast to the more usual type of chronic cholecystitis with sterile bile and persistent infection of the wall.

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## DESCRIPTION OF PLATE

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- FIG. 1. A general view of the gall-bladder wall showing dense lymphocytic infiltration of the mucosa, with lymph-nodule formation.  $\times 20$ .
- FIG. 2. A lymph nodule with hyperplastic germinal center. Polymorphonuclears can be seen just beneath the epithelium and between the epithelial cells.  $\times 200$ .





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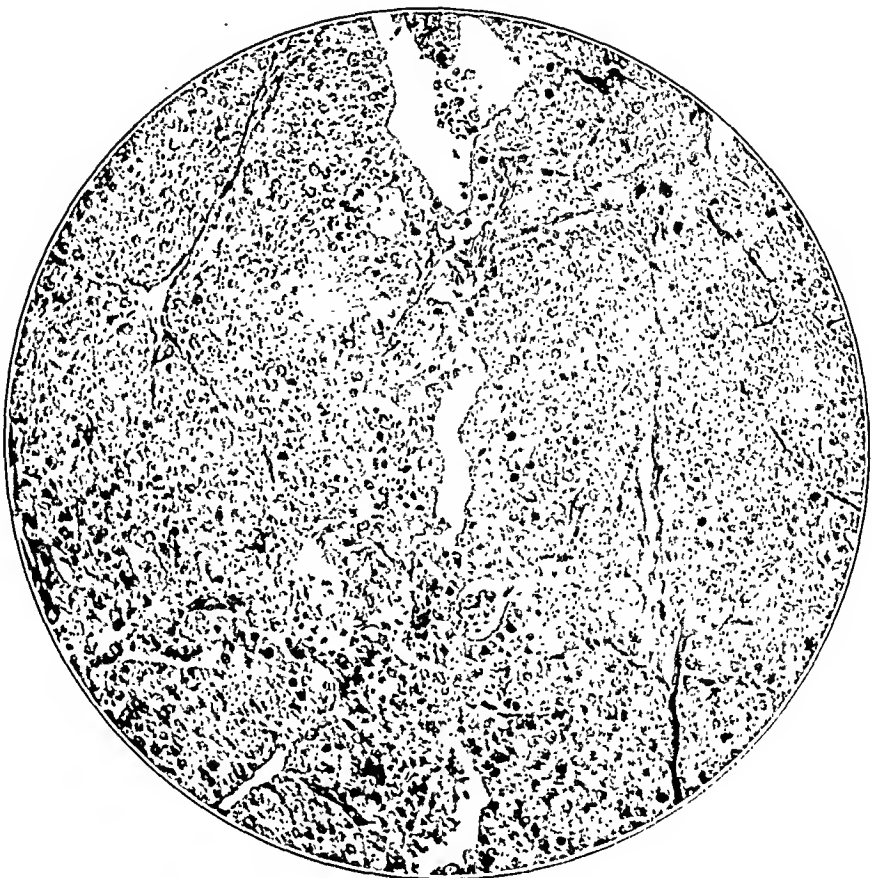
Zeckwer, Isolde T. Spontaneous insulin resistance in rabbits: The effect of thyroidectomy on the response to insulin . . . . .	548*
Zeek, Pearl. See Foot and Zeek . . . . .	605

PLATE 148

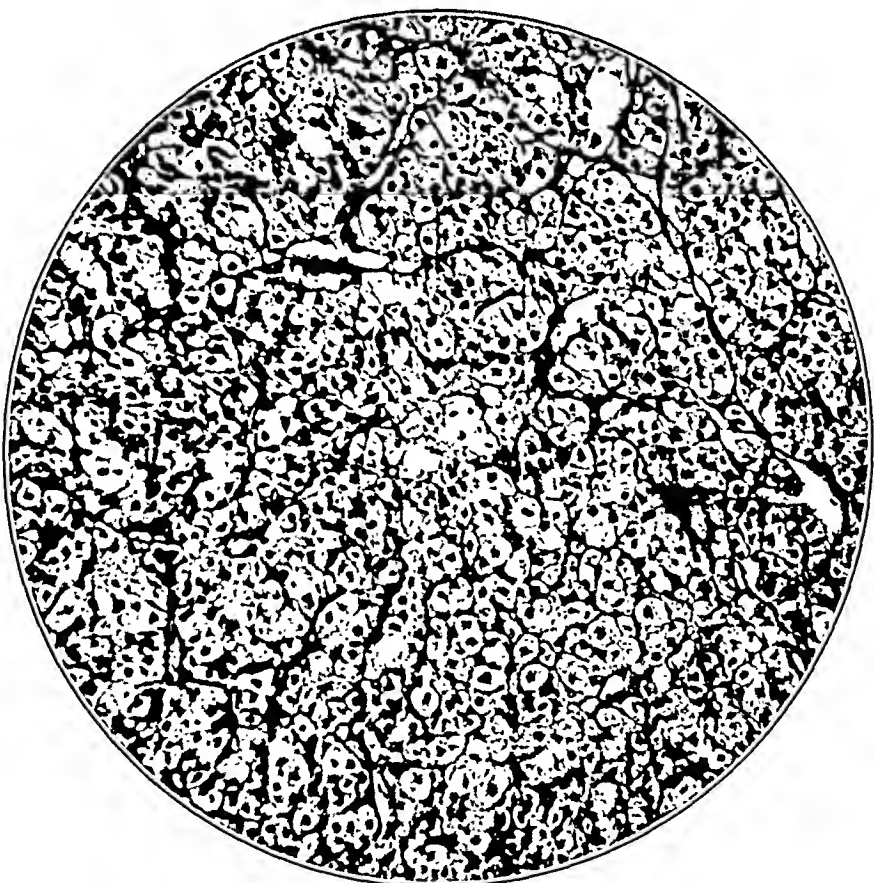
FIG. 15. Case 14. Photomicrograph showing solid and alveoli arrangement of cells.  $\times 130$ .

FIG. 16. Case 14. Note typical Grawitz tumor.  $\times 130$ .





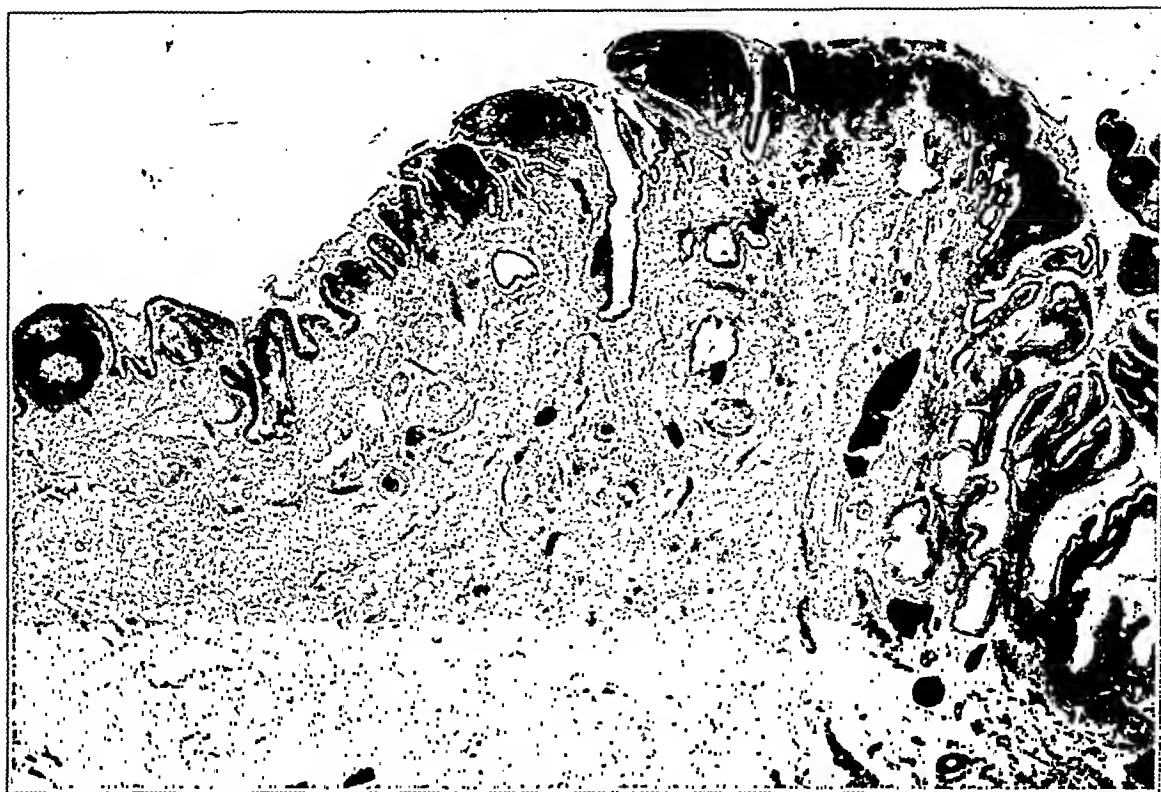
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16

Hansmann and Budd

Massive Unattached Retroperitoneal Tumors



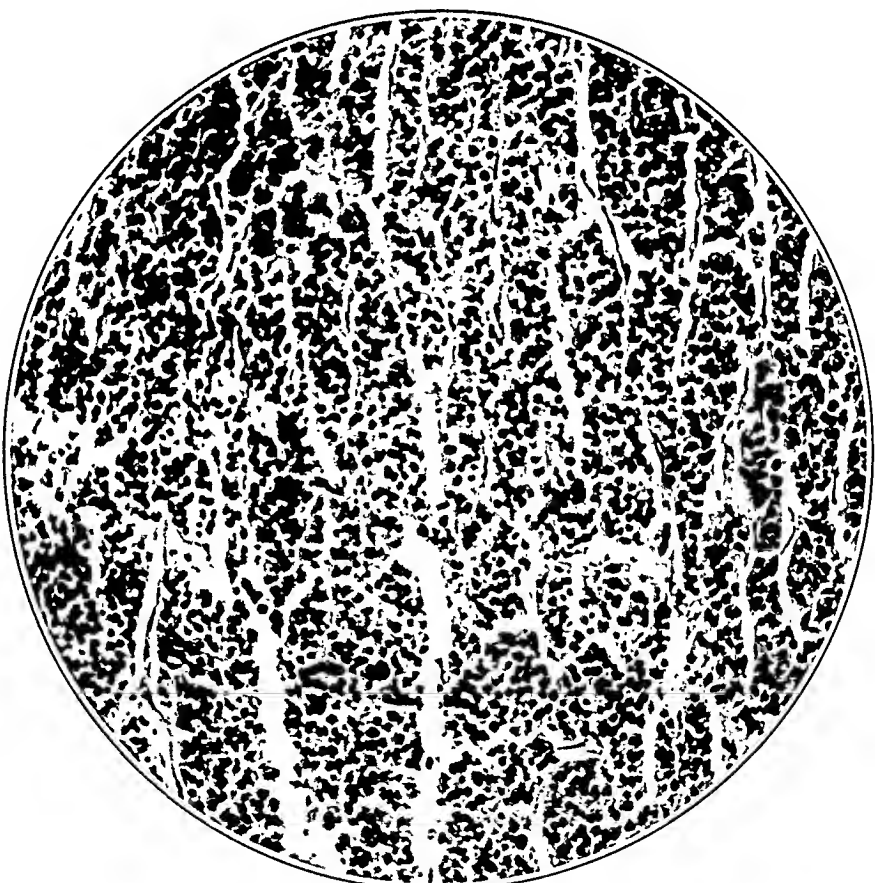
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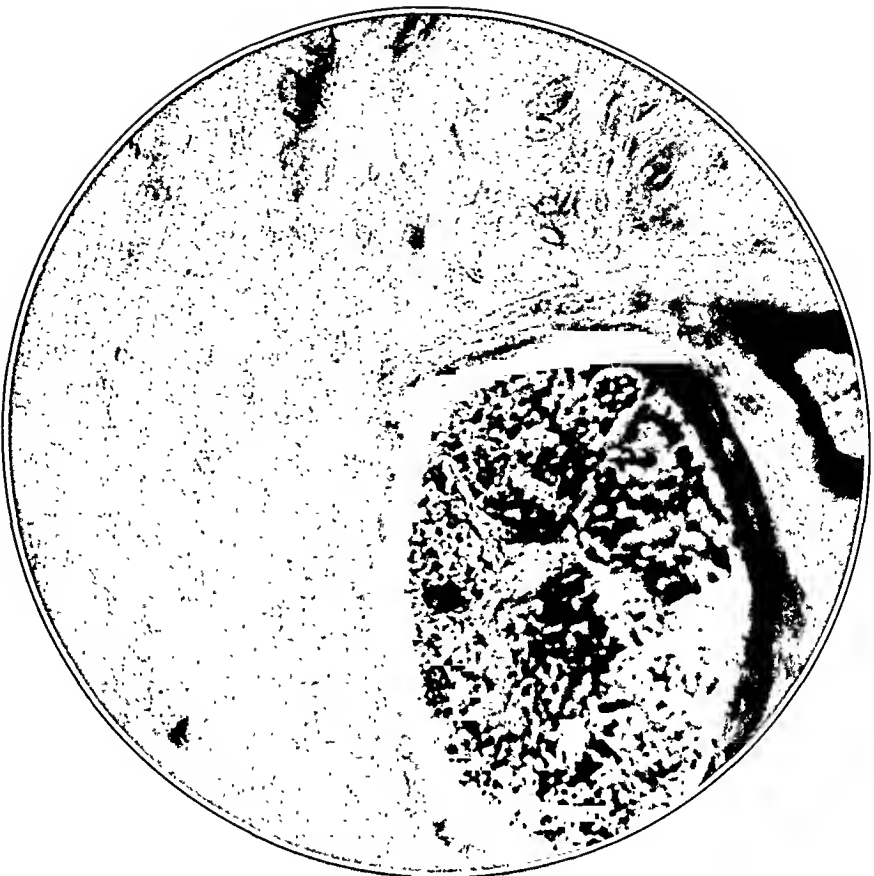


18

PLATE 149

FIG. 17. Case 15. X-ray showing calcified mass high in the left upper quadrant. See Figs. 18 and 19.

FIG. 18. Case 15. Photomicrograph of tumor shown in Fig. 17 showing adrenal-like arrangement of cells.  $\times 130$ .



19



20

PLATE 150

FIG. 19. Case 15. Photomicrograph from same tumor as Fig. 18, showing active bone production.  $\times 130$ .

FIG. 20. Case 16. Note quite accurate reduplication of metanephritic tubules.  $\times 120$ .

## PAPILLIFEROUS TUMORS OF THE THYROID GLAND AND OF ABERRANT THYROID TISSUE \*

ALAN RICHARDS MORITZ AND FRANCIS BAYLESS

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Papilliferous tumors of the thyroid have formed a heterogeneous group, the common feature of which has been gross or microscopic papilliferous epithelial hyperplasia. Such tumors have constituted slightly less than 10 per cent of the malignant epithelial tumors of the thyroid, according to the reports of Müller and Speese, Simpson, Graham and Wilson.

After Wölfler described solid and cystic forms of papilliferous tumors of the thyroid in 1883, little more was done to define the types and to correlate structure with gross characteristics until 1925, when Graham distinguished three kinds of malignant papilliferous tumors. Numerous varieties of tumors have been characterized as papilliferous, and opinions have differed as to their malignancy. Low and Zehbe minimized the significance of papilliferous structure, and because that type of hyperplasia is so common to the thyroid they did not favor distinguishing such tumors from adenocarcinomas. However, Low did observe that papilliferous cystadenomas should be recognized as a type of tumor prone to repeated recurrence, rather than to metastases.

Ehrhardt believed the papilliferous tumors to be a subtype of cylindrical cell carcinoma of the thyroid, but held that the cystic form stood midway between the malignant and benign tumors. This latter opinion was shared by Halstead, Verebly, Müller and Speese, and Plessner, although in some instances the generalization was applied to solid, as well as to cystic forms.

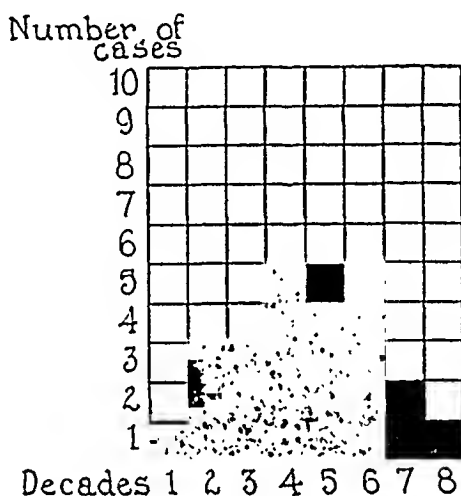
Wegelin recognized a solid and cystic form of true papilliferous tumor, and a papilliferous type of macrofollicular adenoma which he excluded from the papillary group. Simpson did not specify any particular type of papilliferous tumor but agreed with Wegelin that their malignancy was most commonly limited to local invasion or metastasis to neighboring lymph nodes. Neither related the struc-

\* Received for publication June 29, 1931.





tially or entirely filled by macroscopic papillae arising from the lining (Fig. 1). Ten were in the thyroid gland and seventeen in aberrant thyroid tissue. They occurred with slightly greater frequency in males than in females (15:10). The age distribution at operation is shown in Text-Fig. 1. The average preoperative duration of the tumors was fourteen years, in the twelve instances where that information was available. There were no significant differences in age or sex distribution between such tumors arising in the thyroid and those arising in aberrant thyroid tissue. They varied



TEXT-FIG. 1. Age incidence by decades of papilliferous cystadenomas based on age at time of operation or autopsy.

from less than 1 cm. to 10 cm. in diameter, and the largest tumors were in most instances those of longest known duration.

Both unilocular and multilocular cysts were described, and in the latter some of the locules were smooth-lined while others were papilliferous. In such tumors areas of intact or only partially degenerated adenomatous tissue were seen (Fig. 3). Great variation in structure was observed in some of the multiple tumors of aberrant thyroid tissue in which colloid adenomas, cystadenomas and papilliferous cystadenomas coexisted. The papillae varied in size and complexity, were usually covered by cuboidal or columnar epithelium, and were supported on an abundant fibrous stroma, which was commonly the seat of hyaline degeneration and edema, to produce rounding of the free ends (Fig. 1). Although nuclei were seen at the free ends of cells (Langhans) and cells were in some instances cylindrical (Ehrhardt) neither feature was characteristic. Coalescence of papillae

ture of the various types of papilliferous tumors to their gross characteristics. This relation was suggested by Graham who stated that there are three groups of malignant papilliferous tumors: (1) the papilliferous cystadenocarcinoma which is locally malignant; (2) the malignant adenoma exhibiting papilliferous differentiation, which may metastasize widely; and (3) the papilliferous adenocarcinoma not arising in an adenoma, the malignancy of which was not defined.

If the malignancy of these tumors varies with their structure, the significant types should be accurately described. Twenty-eight papilliferous tumors of the thyroid were available for study from the Institute of Pathology of Western Reserve University. There have been at least 149 cases reported, not including the material studied in the various reviews and general treatises in which case protocols were not given. The protocols of seventy-four were considered adequate for the purpose of classification.

The material studied was as follows: Papilliferous tumors of the thyroid gland (twenty-four cases from Lakeside Hospital\* and forty cases from the literature), and papilliferous tumors of aberrant thyroid gland tissue (four cases from Lakeside Hospital and thirty-four cases from the literature). Tumors described in published case reports were classified according to their pathological descriptions and illustrations which, in some instances, were not in accord with the diagnoses given them by their authors.

### PAPILLIFEROUS CYSTADENOMA

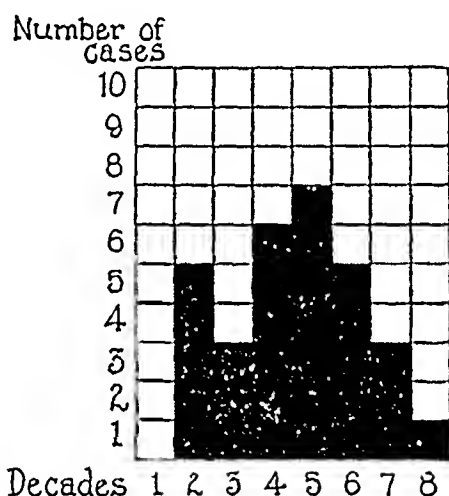
Twenty-seven papilliferous cystadenomas of the thyroid gland or of aberrant thyroid tissue were studied. Twenty-three of them were selected from the literature as having sufficiently detailed pathological descriptions or illustrations for the purpose of classification.† These were encapsulated, cystic tumors which were par-

\* The authors wish to express their appreciation to Doctors Crile, Cutler, Goff, Holloway, Sloan and Weidlein for permission to publish cases operated upon by them, and to Dr. Allen Graham for information regarding the postoperative survival of many of the patients.

† Papilliferous cystadenomas of thyroid gland or of aberrant thyroid tissue included in this study were reported by Binney, Jores, Kapsammer, Kocher (Case 20), Leech, Smith and Clute (Cases 1, 2 and 3), Low (Cases 1, 5 and 6), MacLennan, McGlannan (Cases 2 and 4), Moritz and Bayless (Case 3), Müller and Speese (Case 1), Payr and Martina (Case 1), Plauth, Pollard, Reynier (Case 1), Verebly (Case 4), Wegelin (Case 1), and Zahn and Züllig (Cases 2 and 5).

postoperative X-ray treatment was given. One of these was free from recurrence in six and one-half years and the other in nine years after operation. In some of the so-called recurrences of papilliferous cystadenocarcinomas of aberrant thyroid tissue the possibility of the development of independent new tumors could not be excluded, since multiple tumors were described by Barker, Billings and Paul, Hinterstoisser, Leech, Smith and Clute and were seen in two cases studied by us.

Although these tumors were cystic, there were instances in which the luxuriant growth of papillae in some of the locules and the



TEXT-FIG. 2. Age incidence by decades of papilliferous cystadenocarcinomas based on age at time of operation or autopsy.

scirrhous character of the advancing margin were responsible for the macroscopic appearance of solidity. They were commonly multilocular, and their contents often hemorrhagic. Papillae varied in size and shape, exhibited a tendency to coalesce, and in the more actively proliferating areas were delicate with great multiformity of the covering epithelium. In every case there was capsular invasion, in nine extension into the adjacent cervical muscles or trachea, and in twelve there were metastases to lymph nodes of the neck. In no instance was blood vascular invasion seen. The advancing margin of the tumor was usually characterized by dense fibrosis (Fig. 2) which extended into the adjacent non-tumorous tissue. The invasion was accomplished by the peripheral development of small, solid islands of epithelial cells which formed atypical follicles. Beneath the peripheral zone there was papilliferous infold-

was uncommon. Calcification and lymphoid infiltration of stroma and capsule were common. Follicular differentiation of papilliferous epithelium was uncommon and portions of such tumors comprised of colloid-containing follicles were regarded as rests of the original non-papilliferous adenoma. Usually the contents of the cysts were thin and sanguinous.

The transitional forms seen in multiple tumors of aberrant thyroid tissue suggested that this type of neoplasm developed in adenomas which were the seat of cystic degeneration.

### PAPILLIFEROUS CYSTADENOCARCINOMA

Thirty-five papilliferous cystadenocarcinomas of the thyroid gland, or of aberrant thyroid tissue were studied. Twenty-seven of these were selected from the literature as having sufficiently detailed pathological descriptions or illustrations to permit of classification.\* These malignant tumors were cystic and papilliferous in the gross and the only essential differences between them and the benign papilliferous cystadenomas were their invasive growth and metastasis to cervical lymph nodes. In twenty cases the tumors originated in the thyroid gland, and in fifteen in aberrant thyroid tissue. The age at time of operation is shown in Text-Fig. 2.

As in the papilliferous cystadenomas there was a greater number of cases operated upon in the fourth, fifth, and sixth decades than in any other three decades, but the difference was not marked and here, as in the preceding group, the long preoperative existence of the tumors indicated an earlier age onset than is shown in Text-Fig. 2. The preoperative duration of the tumors was about the same as that of the papilliferous cystadenomas. Twenty-four were in females and six in males.

Follow-up observations were available on fourteen patients. Eight of them showed recurrences in three years or less, but in six there were no recurrences in from three to nine years. In two cases the tumors were large, the operations were deemed palliative, and

\* Papilliferous cystadenocarcinomas of the thyroid or of aberrant thyroid gland tissue included in this study were reported by Barker, Berger, Billings and Paul, Caranza, Ehrhardt (Case 1), Günzler, Hinterstoisser (Case 3), Hueck (Case 5), Hughes (Cases 2 and 6), Kamsler, Langhans (Cases 1, 2 and 3), Leech, Smith and Clute (Case 4), Moritz and Bayless (Cases 5 and 6), Payr and Martina (Case 3), Plessner, Pool, Rühl, Simpson, Schrager, Smoler, Verebly (Cases 2 and 3), Wegelin (Cases 3 and 5), and Züllig (Case 1).



ing into the small follicles (Fig. 2 and Fig. 7). As the center of the tumor was approached the follicles were larger and the papillae more luxuriant, until there was a final coalescence with the major cyst cavity, or contribution of new locules to it. Transitional stages were seen between papilliferous cystadenoma and papilliferous cystadenocarcinoma. Arbitrary distinction depended upon the presence or absence of transcapsular infiltration.

That the papilliferous cystadenocarcinomas developed from cystadenomas seemed probable because of the presence of transitional forms and also because in cases having multiple tumors of aberrant thyroid tissue the coexistence of cystadenoma, papilliferous cystadenoma and papilliferous cystadenocarcinoma was observed. The metastases were papilliferous and the larger ones were cystic. The papillae developed in small atypical follicles and there was evidence of secretory activity with distention of the follicles to cystic proportions.

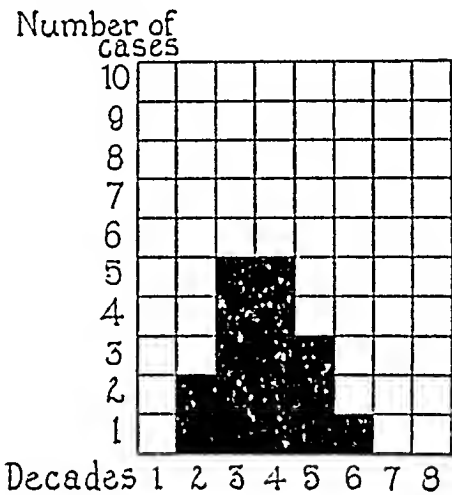
### PAPILLIFEROUS ADENOMAS

Seventeen papilliferous adenomas of the thyroid gland or of aberrant thyroid tissue were studied, twelve of which were selected from the literature.\* Fifteen developed in the thyroid gland and two in aberrant thyroid tissue. Fourteen were in females and three in males. The age incidence at time of operation is seen in Text-Fig. 3. These tumors, three of which were included in Wölfler's original description of papilliferous newgrowths of the thyroid, were not primarily papilliferous, as were the papilliferous cystadenomas or cystadenocarcinomas. The papilliferous character was microscopic and was due to diffuse or focal intra-acinar epithelial hyperplasia. It was similar to that commonly seen in association with Graves' disease and did not go on to the formation of cysts filled with macroscopic papillae.

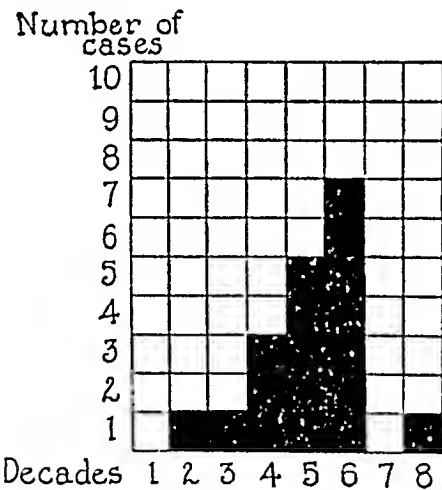
Martini described a tumor in which there was a subcapsular zone of cystic follicles into which the epithelium extended in folds and papilliferous excrescences, but both his description and illustrations indicated that the change was not truly papilliferous, but simple

\* Papilliferous adenomas of thyroid have been reported by Kocher (Case 19), Langhans (Case 5), Low (Cases 2 and 4), Martini, Moritz and Bayless (Case 4), Verebly (Case 1), Wegelin (Case 4), Wischmann, Wölfler (Cases 1, 2 and 3), and Züllig (Case 4).

hyperplasia. Papilliferous hyperplasia into cystic follicles was seen in Wölfler's Cases 1 and 3, and Züllig's Case 4. The papillae of such tumors were small, relatively simple, and covered by uniform columnar epithelial cells. They were supported by a delicate stroma which did not exhibit degenerative changes. Calcification, hemorrhage,



TEXT-FIG. 3. Age incidence by decades of papilliferous adenomas based on age at time of operation.



TEXT-FIG. 4. Age incidence by decades of malignant papilliferous adenomas based on age at time of operation or autopsy.

lymphocytic infiltration and fibrosis were not characteristic, although Wischmann described such a tumor in which there was so much calcification that he chose to call it an "adenoma cylindrocellulare papilliferum psammomatosum." In no instance was there capsular or vascular invasion, or metastasis.

## PAPILLIFEROUS MALIGNANT ADENOMAS

Nineteen papilliferous malignant adenomas were studied, twelve of which were from the literature.\* These were solid, more or less completely encapsulated tumors in which papilliferous hyperplasia was usually microscopic and consisted focal inof diffuse or trafollicular epithelial proliferation. Malignancy was manifested by invasion of capsule, or by blood or lymph vascular metastases. Sixteen of the tumors originated in the thyroid gland and three in aberrant thyroid tissue. Eleven of the patients were females and seven were males. The age incidence at time of operation or autopsy is shown in Text-Fig. 4.

In this group the clinical histories usually indicated the presence of a goiter or tumor many years prior to operation or death, with a recent preoperative or ante mortem acceleration in growth. In three instances the tumors were so large and showed such extensive local invasion that origin in an adenoma could not be identified. In several of the tumors there were macroscopic cystic areas, usually degenerative in character and not sharply defined. With few exceptions, these tumors displayed papilliferous structures similar to those of the preceding group (papilliferous adenomas), (Fig. 6). Portions of the tumors in which follicular structure was manifest exhibited intrafollicular papilliferous hyperplasia of epithelium. The advancing margins were not always papilliferous. The case of McCarthy and Karsner was an exception, in that the pulmonary metastases, as well as the primary tumor, were papilliferous and the papillae were not of the simple delicate variety characteristic of simple hyperplasia, but were stout arborescent structures with abundant stroma and focal calcification. In one of our cases the tumor was predominantly adenocarcinoma although there were true papilliferous areas, and in one of the intravascular tumor masses papilliferous differentiation was apparent. The tumor reported by Wohl cannot be unequivocally diagnosed a papilliferous malignant adenoma. In four instances the metastases were papilliferous (Kocher, Halstead, Hudson, McCarthy and Karsner).

\* Such tumors have been reported by Fedeli (Cases 1 and 3), Halstead (Cases 2 and 3), Hudson, Kocher (Cases 14 and 15), Langhans (Case 4), McCarthy and Karsner, Wegelin (Case 7), Wohl, and Zehbe.



In four of the seven cases studied by the authors, and in five of the twelve cases from the literature, malignancy was manifested by blood vascular invasion. Local lymph nodes were involved in six instances. Of the eight patients followed, four were living and well in six years or more, one was dead in six years, one in three and one-half years and two lived less than a year.

## PAPILLIFEROUS CARCINOIDS

### *Case Reports*

CASE 1. A white male, 54 years of age, gave a history of goiter for three years with symptoms of Graves' disease for two years. Thyroidectomy was performed. In the lower pole of the right lobe there was a small, pale, scirrhous lesion which measured about 1.5 cm. in diameter, not encapsulated, but sharply defined. Its cut surface was gray, uneven, gritty and of non-uniform density. Microscopically, it was comprised of discrete but atypical and often elongated, non-colloid-containing acini distributed through an abundant, dense fibrous stroma. The stroma was continued beyond the parenchyma and intervened between it and the adjacent gland tissue. There was calcification and diffuse lymphocytic infiltration of the stroma. Some of the follicles were large and many of them were filled by one or several branching papillae which were covered by single or multiple layers of epithelium which varied greatly in size and shape of cells and nuclei. The surrounding gland tissue was colloid-containing and nodular. The patient was living and well six and one-half years after operation.

CASE 2. White female 52 years of age. Goiter since puberty. A large (115 gm.) nodular goiter was removed. In the upper pole, in the non-tumorous portion of the gland, there was a small, pale, densely fibrous area which was of non-uniform density and structure and which contained no colloid (Fig. 4). This lesion was of irregular outline, and its surface merged with the fibrous stroma of the surrounding colloid-containing gland tissue without sharp distinction. One surface was attached to, but had not invaded the capsule. Microscopically this area consisted of atypical, labyrinthine acini into which there was papilliferous invagination of lining epithelium. The papillae were both simple and complex and showed some secondary acinar differentiation. The epithelial cells lining the acini and

covering the papillae were larger, more acidophilic and manifested greater multiformity of cells and nuclei than in the adjacent colloid-containing thyroid gland. There were occasional mitoses and nuclear monstrosities. Numerous small islands of undifferentiated epithelium were distributed through the stroma which was abundant, densely fibrous, diffusely infiltrated by lymphocytes and which extended out into and merged with the interstitial tissue of the adjacent gland. The small solid nests of epithelium showed advanced degenerative changes and atrophy, as though being replaced by the proliferating stroma. The patient was living and free from recurrence thirteen years after operation.

CASE 3. White female, 29 years of age. Graves' disease for about two years. Right lobectomy was performed. The lobe contained two adenomas and was generally involuted with some diffuse increase in fibrous connective tissue. At the upper pole, attached to but not invading the capsule, was an irregularly shaped, non-encapsulated mass 7 mm. in diameter, which was pale, fibrous and gritty on section. Microscopically, small and large compressed atypical acini were distributed through a dense, fibrous stroma. There was intra-acinar proliferation of simple and branching papillae. Both acinar and papilliferous epithelium were atypical and differed from epithelium of surrounding thyroid in size and staining qualities of cells. The cells were large, polymorphous, and many exhibited degenerative changes. The stroma was abundant, the seat of focal calcification, and diffusely infiltrated by lymphocytes and large mononuclear cells. The patient was living and well fifteen and one-half years after operation.

CASE 4. The lesion was an incidental finding in an adenomatous goiter, the clinical data concerning which were not available. There was a small scirrhous, subcapsular mass which was not encapsulated but was sharply differentiated. This mass resembled the lesions described in Cases 1, 2 and 3 and was about 1 cm. in its maximum diameter. It manifested greater degenerative changes with desquamation of degenerate acinar epithelial cells and compression of the atypical acini by the actively proliferating fibrous connective tissue.

In these four cases the lesion of the thyroid corresponded to that described by Graham as "adenocarcinoma not arising in an adenoma." The diagnosis of "papilliferous carcinoid" is not intended to imply that all such lesions are papilliferous, or that there is no

point out that infectious cirrhosis and obstructive cirrhosis should not be considered as a single entity but that they differ in respect to etiology, pathological anatomy and in their clinical manifestations, and (3) to show how these two conditions may occur in combination — in which case the pathological and clinical manifestations would depend upon which of the two processes was playing the leading rôle.

### LITERATURE

In a paper in 1876 by Charcot and Gombault<sup>1</sup> the term biliary cirrhosis was used to describe lesions of the liver associated with stones in the Ampulla of Vater, cancer of the head of the pancreas and infectious cholangitis. As no attempt at that time was made to differentiate these conditions, the term "biliary cirrhosis" (we adopt Weber's definition<sup>2</sup>) as meaning "any cirrhosis of the liver originating from diseases of the bile ducts or obstruction to the out-flow of bile," is quite acceptable.

Heineke<sup>3</sup> in 1897 differentiated two distinct types of biliary cirrhosis; first, one arising from an inflammatory reaction within and around the small bile capillaries and extending outward into the periphery of the lobule, the process being infectious and of an ascending nature. The second type was based on prolonged biliary obstruction.

Mallory<sup>4</sup> in 1911 described infectious cirrhosis as being a type of cirrhosis of the liver resulting from an infection within and around the smaller bile ducts, which corresponds to the infectious type of biliary cirrhosis reported by Heineke.

Rolleston<sup>5</sup> expressed little doubt that the factor which determines fibrosis about the intrahepatic ducts in gall stone obstruction is infection, and regarded the microscopic findings in cases of mechanical biliary obstruction as being of neither clinical nor pathological significance.

### MATERIAL FOR STUDY

In a study of the livers from several thousand autopsies performed at the Boston City Hospital, together with a large quantity of material sent in from other hospitals, a total of sixty-five cases was collected showing lesions within the liver which could be grouped as types of "biliary cirrhosis," using again Weber's defi-

## INFECTIOUS CIRRHOSIS\*

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Infectious cirrhosis may be regarded as one of the less common diseases of the liver. It is rare to find it in a pure uncomplicated form. More often it occurs in combination with the obstructive type of cirrhosis. The two types, namely the infectious and the obstructive, occurring either alone or together are often considered as a common entity and grouped together with certain other diseases of the liver under the rather confusing and much misused term of biliary cirrhosis.

It is not the intention of this paper to describe a new and original entity. Infectious cirrhosis is not a new disease: its signs and symptoms as well as its pathological anatomy have long been recognized. One finds, however, in practice, and also in text-books of medicine and pathology considerable uncertainty and misinterpretation in the use of this term. Such confusion may be explained in several ways. First, the disease is not common, consequently the clinician seldom has a fair opportunity to study it; second, the clinical manifestations are rather variable, indeed may be so vague or so slight that an infectious process within the liver is often unsuspected. Similarly, the pathologist who may recognize the disease when seen in its well advanced stages, may overlook it in its earliest form or fail to interpret the varied lesions that one sees in the acute, healing, chronic and healed stages, as nothing more or less than different phases of a single disease. In addition to these facts, one finds the view still prevailing that the terms infectious cirrhosis and obstructive cirrhosis are simply two names for a similar entity; that uncomplicated and prolonged obstruction to the outflow of bile will produce neither recognizable nor constant histological changes; that prolonged biliary obstruction is always complicated with infection within the bile ducts, and that any gross or histological findings that are to be seen within the liver are only the natural result of an inflammatory reaction incited by some form of bacterium.

The purpose of this paper is (1) to define infectious cirrhosis, and to describe it from both the pathological and clinical aspects; (2) to

\* Received for publication July 8, 1929.

## HISTOLOGICAL FEATURES OF INFECTIOUS CIRRHOSIS

The picture at first is merely that of acute inflammation within the portal areas, resulting from the presence of pathogenic organisms within the terminal bile ducts; from here the inflammatory reaction spreads among the surrounding liver cells (Fig. 1) so that the lesion is confined not alone to the portal areas but also to the peripheral zones of the lobules.

The portal areas which appear to suffer the brunt of the infection are the rather small or medium sized ones; however, even the smallest and largest may at times become involved. Furthermore, the inflammatory reaction may not be equally advanced in all portal areas within the liver so that some may show acute inflammation, others a rather chronic reaction, whereas in others the process may be almost or entirely healed. In order to interpret the histological findings in a liver in which the process has entirely healed, it is necessary to have traced and to know thoroughly the development of the lesion from its early beginning to its end.

The small bile duct is the center of an acute inflammatory reaction; the lumen is distended with polymorphonuclear or endothelial leucocytes (Fig. 4), depending on the number and virulence of the infecting organism. The epithelial cells are stretched and show regressive changes, and the surrounding stroma is infiltrated with an acute inflammatory exudate. As the organisms invade the tissue around the ducts (Figs. 2 and 3) and the process extends, the liver cells in the outer portions of the lobules show retrograde changes; even at this early stage one may find proliferation of fibroblasts. Later, there is a zone of degenerating and necrotic liver cells infiltrated with polymorphonuclear and endothelial leucocytes surrounding the portal area. The endothelial cells lining the sinusoids, and the supporting connective tissue are also damaged so that the normal architecture of the periphery of the lobules is entirely lost.

Very early, the bile duct epithelium begins to proliferate, mitoses are numerous, and any small periportal ducts that have not been destroyed, elongate and give rise to many new ducts which extend out into the surrounding zone of necrosis to link up with the free ends of bile capillaries.

Occasionally one finds the walls of vessels also involved in the in-

nition. Five of this group could be classified as uncomplicated infectious cirrhosis; thirteen were cases of combined infectious and obstructive cirrhoses; thirty-nine were cases of uncomplicated obstructive cirrhosis; while the remaining eight were cases of obstructive cirrhosis that had terminated fatally with an acute suppurative cholangitis with abscesses in the liver. Since this last group showed no cirrhotic changes of infectious origin, they were not included in this series.

### ETIOLOGY OF INFECTIOUS CIRRHOSIS

As the name implies, this type is due to an infection within the terminal bile ducts which invades the adjoining lobules. Cultures of the liver were taken in only two of the five cases and although the colon bacillus was isolated in pure culture from each of these, such evidence is quite insufficient to ascribe to it any definite etiological rôle. However, in one of these two cases microscopic examination showed rather small slender bacilli in the lesions, whereas in the other no organisms could be demonstrated although the lesions had not passed the acute inflammatory stage. Of the three remaining cases, two showed only old healed lesions; therefore, it is not surprising that organisms could not be found. In the last of the five cases, slender bacilli could be demonstrated within the chronic inflammatory lesions. Unfortunately, this case had not been cultured.

A temporary obstruction to the outflow of bile may possibly have been an important contributing factor in the etiology of these five cases, because in each there was a history of a transient, though slight degree of jaundice. However, at autopsy no obstruction was found and a histological examination of the liver showed no lesion suggesting biliary obstruction.

The manner in which organisms reach the terminal bile ducts is probably by an ascending course from the duodenum through the large and small bile ducts. However, the possibility of infection reaching the liver by way of the circulation and lymphatics cannot be entirely disregarded. This phase of the problem has recently been fully reviewed by Brulé<sup>6</sup> who discusses in detail all the possible paths by which infection may reach the liver.

is so little grossly to suggest the histological changes that the lesion at this stage may be readily overlooked.

As the process becomes more chronic and more extensive the liver enlarges from a third to double its normal size. The capsule is smooth and tense. The consistence is increased and the liver cuts with more resistance. The fresh surface may be a little bile-stained or deeply congested, but the broad portal areas even at this stage are not very clearly defined.

The healed stage is characterized by a contraction of the newly formed stroma so that once again the liver may return to about normal size. The surface is rather uniformly and finely nodular with the nodules sometimes measuring nearly one centimeter in diameter. The consistence is firm and the liver cuts with greatly increased resistance. The cut surface presents a striking picture (Fig. 5). There is a marked increase in the amount of connective tissue about the portal areas so that they appear as grayish white, fibrous bands which may be traced from the transverse sinus to the capsule.

### CLINICAL ASPECTS OF INFECTIOUS CIRRHOSIS

Since these are so dependent on the nature of the lesion within the liver, they will of course vary considerably. The onset may be gradual and unaccompanied by any diagnostic sign or symptom; however, as is so commonly the case, jaundice, perhaps very slight and only transient, is one of the first things to be noticed. Lassitude, drowsiness and gastro-intestinal disturbances, accompanied later by loss of strength and weight are clinical findings common to each case. The temperature may vary slightly from day to day but seldom rises above 100 F. Similarly, the blood count may show no appreciable change, except a slight leucocytosis which sometimes reaches twelve or fifteen thousand cells per cubic millimeter.

In the early stage, as already pointed out, the liver is normal in size; in the chronic and healing stages it is enlarged, while later, if recovery ensues, it may shrink again to normal size and is then uniformly nodular. The spleen may be somewhat enlarged from the onset. As the process in the liver regresses, with a gradual contraction of the newly formed connective tissue, the portal circulation becomes appreciably obstructed so that in the healed stages the spleen may

flammatory reaction and fibrin thrombi adherent to the damaged endothelium partially obstructing the lumina. The lymphatics in the capsule and large portal areas are filled with endothelial and polymorphonuclear leucocytes, coagulated albumin and fibrin.

In the healing stage, when the process is less active and the bacteria have died out, the exudate consists chiefly of endothelial leucocytes, many of which are phagocytic and contain necrotic cellular débris. Long before this period, as already mentioned, fibroblasts have begun to proliferate, largely as the result of direct injury to the connective tissue by the bacteria and their toxins, but also in part to form stroma for the greatly increased number of small bile ducts.

In the smaller portal areas the lesion spreads very uniformly as an expanding circle from the bile duct toward the surrounding hepatic veins, but in the larger portal areas, where there may be several ducts and where the ducts are so eccentric, the infection may not reach each of the adjacent lobules but may extend out in a semicircular fan-shaped manner destroying the periphery of only one or more lobules along one side. On the whole, however, the true lobular arrangement is more or less perfectly preserved and the portal areas, as a result of the extensive exudate, of the necrosis of the liver cells and of the encroachment and proliferative activity on the part of the terminal bile ducts and connective tissue, show up as broad bands running rather regularly among the lobules of liver cells.

In contrast to the lesion in the acute or healing stages, the healed lesion is much less conspicuous. The necrotic cells have been removed; a few scattered lymphocytes, singly or in foci, are all that remain of the inflammatory exudate; the stroma is shrunken and the small bile ducts are compressed. The portal areas now stand out simply as bands of dense fibrous tissue containing many small bile ducts. The portal vessels are frequently sclerosed, and here and there, lying near the portal areas, are small isolated clumps of liver cells.

#### ANATOMICAL FEATURES OF INFECTIOUS CIRRHOSIS

In the early stage of infectious cirrhosis, the liver is normal in size and contour, the capsule is smooth, the fresh surface may be bile-stained and the portal areas may be slightly accentuated, but there



## INFECTIOUS CIRRHOSIS COMBINED WITH OBSTRUCTIVE CIRRHOSIS

This combined form of cirrhosis, though much less common than the simple obstructive type, is encountered more frequently than the infectious type alone, and is simply the result of an infection within the terminal bile ducts of a liver that has already begun to show the characteristic changes resulting from prolonged biliary obstruction. Hence, the lesion is merely the picture of one type of cirrhosis superimposed on another, and because the inflammatory process resulting from the infection spreads quickly it soon becomes the outstanding feature both grossly and microscopically (Figs. 6, 7 and 8).

This combined form of cirrhosis may occur at any age; two examples were in infants several months old, one in an adolescent, and the remainder in adults. In our small series males and females were about equally affected. As in the simple uncomplicated type of infectious cirrhosis, the infection probably reached the liver by way of the biliary system; however, since two of our cases were in infants that at the time of the autopsy were considered to show complete atresia of the common bile duct, the possibility of the infection coming from the vascular or the lymphatic system should also be considered. The infecting organism in three of the cases that were cultured was the colon bacillus; unfortunately, no bacteriological studies were carried out in the others, but in eight, bacilli were demonstrable histologically within the lesions.

At this point it is quite unnecessary to repeat the description of the histological lesions of both the obstructive and infectious types of cirrhosis and consequently only the most characteristic findings will be mentioned. The lesions of infectious cirrhosis may be in the acute or chronic stage, but since this combined type of cirrhosis almost invariably ends fatally, a liver showing the combined healed infectious and obstructive lesions is extremely rare and was encountered in only one of our cases, and in this the obstructing agent had disappeared.

One of the remarkable features of this combined type of cirrhosis is the large number of infarcts seen in the periphery of the lobules which resemble in every way those that are occasionally found in the uncomplicated type of obstructive cirrhosis. Sooner or later many of these become infected and are then distended with an inflamma-

be considerably enlarged, due to chronic passive congestion. Ascites and esophageal varices were found in one case. In the healed stage there is neither jaundice nor histological evidence of bile stasis.

### SUMMARY OF THE PATHOLOGICAL FINDINGS IN INFECTIOUS CIRRHOSIS

The outstanding features of infectious cirrhosis can be stated briefly; a sequence of changes may be followed beginning with an infection within the terminal bile ducts, which soon spreads and destroys the surrounding liver cells. This not infrequently causes damage to the walls of the vessels as well. Bile ducts and connective tissue rapidly regenerate and, with the disappearance of the inflammatory exudate and the necrotic cellular débris, the late characteristics of this type of cirrhosis soon become well established.

### COMPARISON BETWEEN INFECTIOUS AND OBSTRUCTIVE CIRRHOSES

First as regards etiology; one results from an infection within the terminal bile ducts, the other from prolonged biliary obstruction. The infectious type of cirrhosis lacks a uniform general distribution, whereas in the obstructive type of cirrhosis<sup>7</sup> the histological changes characterized by an elongation and apparent increase in bile ducts are found in every portal area within the liver. Furthermore, whereas the outstanding features in the early stage of infectious cirrhosis are infection, inflammation and necrosis, in the obstructive type it is the extreme degree of bile stasis which first attracts attention.

Grossly, the liver in uncomplicated cases of obstructive cirrhosis is normal in size, and the surface is smooth except when the obstruction has been of very long standing, and then it assumes a very finely granular Morocco leather appearance.

Lastly, while the classical signs of a severe degree of obstruction to the portal circulation, namely esophageal varices, a large spleen and ascites, are found in long-standing and healed cases of infectious cirrhosis, they are lacking in cases of the obstructive type.

2. Infectious cirrhosis is contrasted with obstructive cirrhosis from the pathological and clinical aspects.

3. The literature bearing on infectious cirrhosis is briefly reviewed.

I am indebted to Dr. F. B. Mallory for his help and criticism and for the illustrations.

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## DESCRIPTION OF PLATES

### PLATE 14

FIG. 1. Periphery of a lobule, showing invasion by colon bacilli, destruction of liver cells and an inflammatory exudate of polymorphonuclear and endothelial leucocytes. The periportal connective tissue is increased in amount. From a man aged 85 years.  $\times 250$ .

FIG. 2. Invasion of liver parenchyma by colon bacilli: acute inflammatory exudate consisting of polymorphonuclear leucocytes. From the same case as Fig. 1.  $\times 1000$ .

tory exudate which compresses the surrounding liver cells. The origin of these areas of necrosis,<sup>8</sup> which occur rarely in simple obstructive cirrhosis and more commonly in the combined form of obstructive and infectious cirrhosis, can be explained on several grounds. First, there is a mechanical interference with the blood supply in these areas as a result of wide distention of the bile ducts compressing the vessels in the portal area; (2) in the process of inflammation there is a slowing of the circulation, and (3) as a result of the injurious agent the vascular endothelium is injured and thrombi are frequently formed. With this explanation these areas of necrosis may be interpreted simply as infarcts which have undergone unusually rapid liquefaction as a result of imbibition with bile.

Another finding in this combined form of cirrhosis is the relatively inconspicuous rôle bile stasis plays in the picture, so that instead of seeing the bile capillaries distended with bile they are collapsed and almost empty. This may probably be explained either by the escape of bile from the free ends of the bile capillaries into the periportal zone of inflammation and necrosis, or on the basis that the liver cells, in the presence of infection, cease to carry out their secretory function, and consequently bile is not being formed.

### COMMENT

Uncomplicated infectious cirrhosis is one of the rarer diseases of the liver. It is caused probably in most instances by the colon bacillus, and is characterized by a variable degree of connective tissue and bile duct proliferation about the portal areas.

Obstructive cirrhosis resulting from prolonged biliary obstruction is a much more common condition although less conspicuous and hence frequently overlooked.

Most cases of infectious cirrhosis are found in combination with obstructive cirrhosis, but even this combined type is relatively rare and is almost invariably fatal.

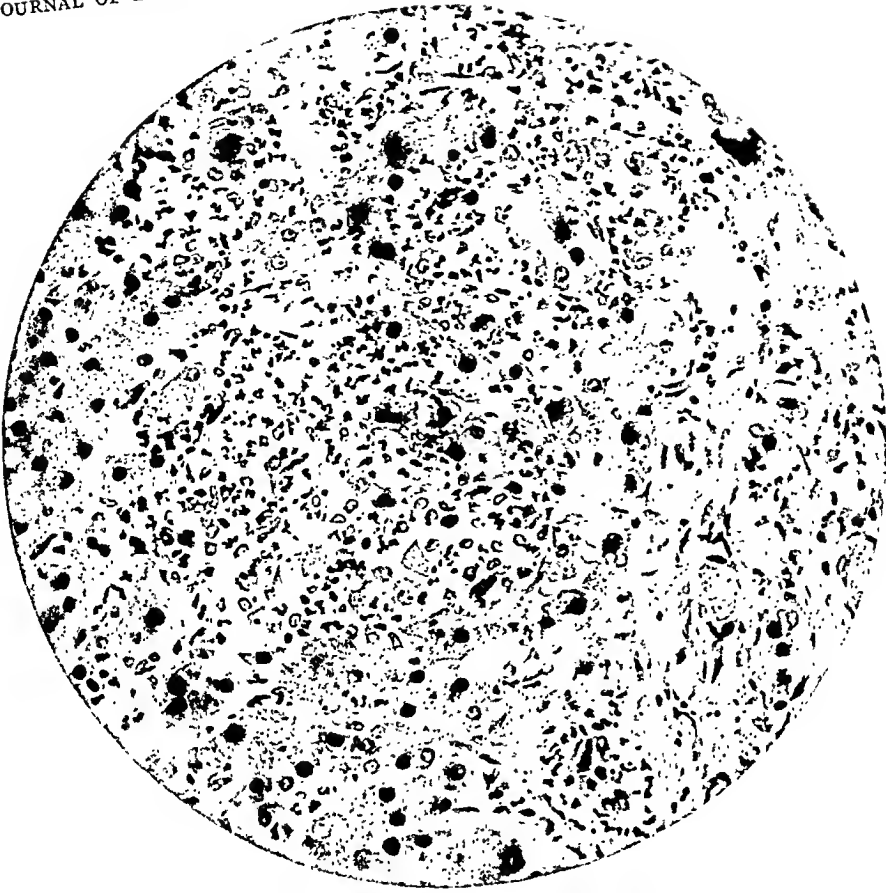
### SUMMARY

1. The gross and histological lesions of infectious cirrhosis, when occurring alone or in combination with obstructive cirrhosis, are described.

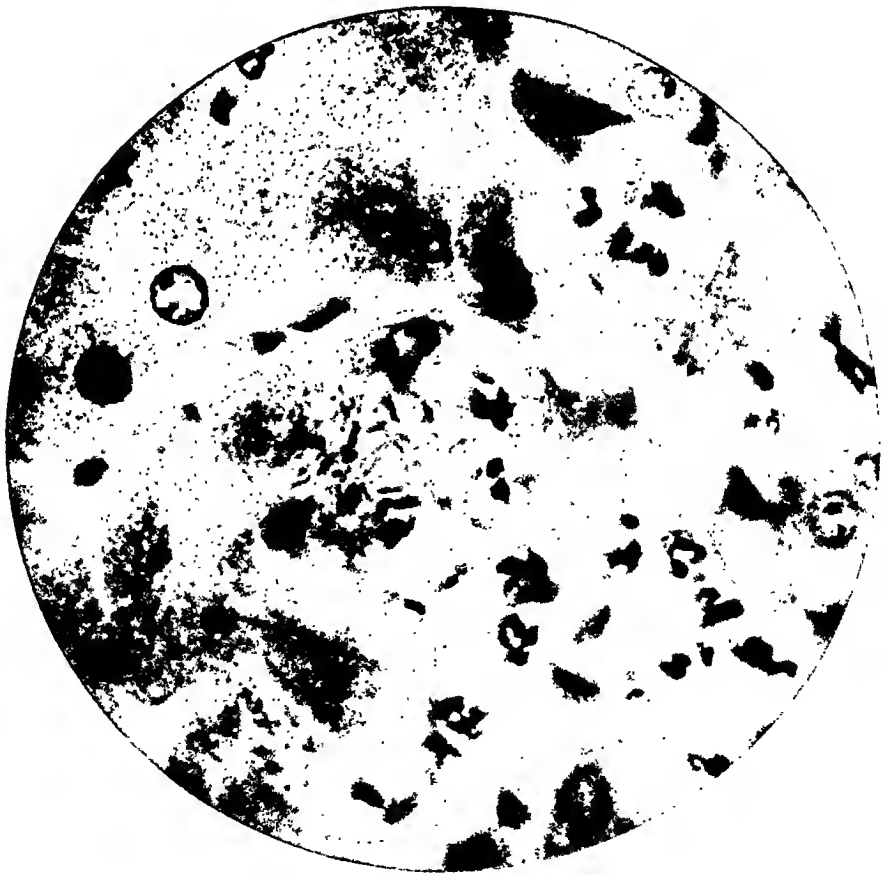
PLATE 15

FIG. 3. Invasion of liver parenchyma by colon bacilli: acute inflammatory exudate consisting of polymorphonuclear and endothelial leucocytes. From the same case as Figs. 1 and 2.  $\times 2000$ .

FIG. 4. Bile duct dilated: contains an irregular cylindrical mass of inspissated bile and an exudate of endothelial leucocytes. The surrounding stroma is infiltrated by endothelial and polymorphonuclear leucocytes and a few lymphocytes. From a girl 13 years of age whose liver was twice normal size.  $\times 1000$ .



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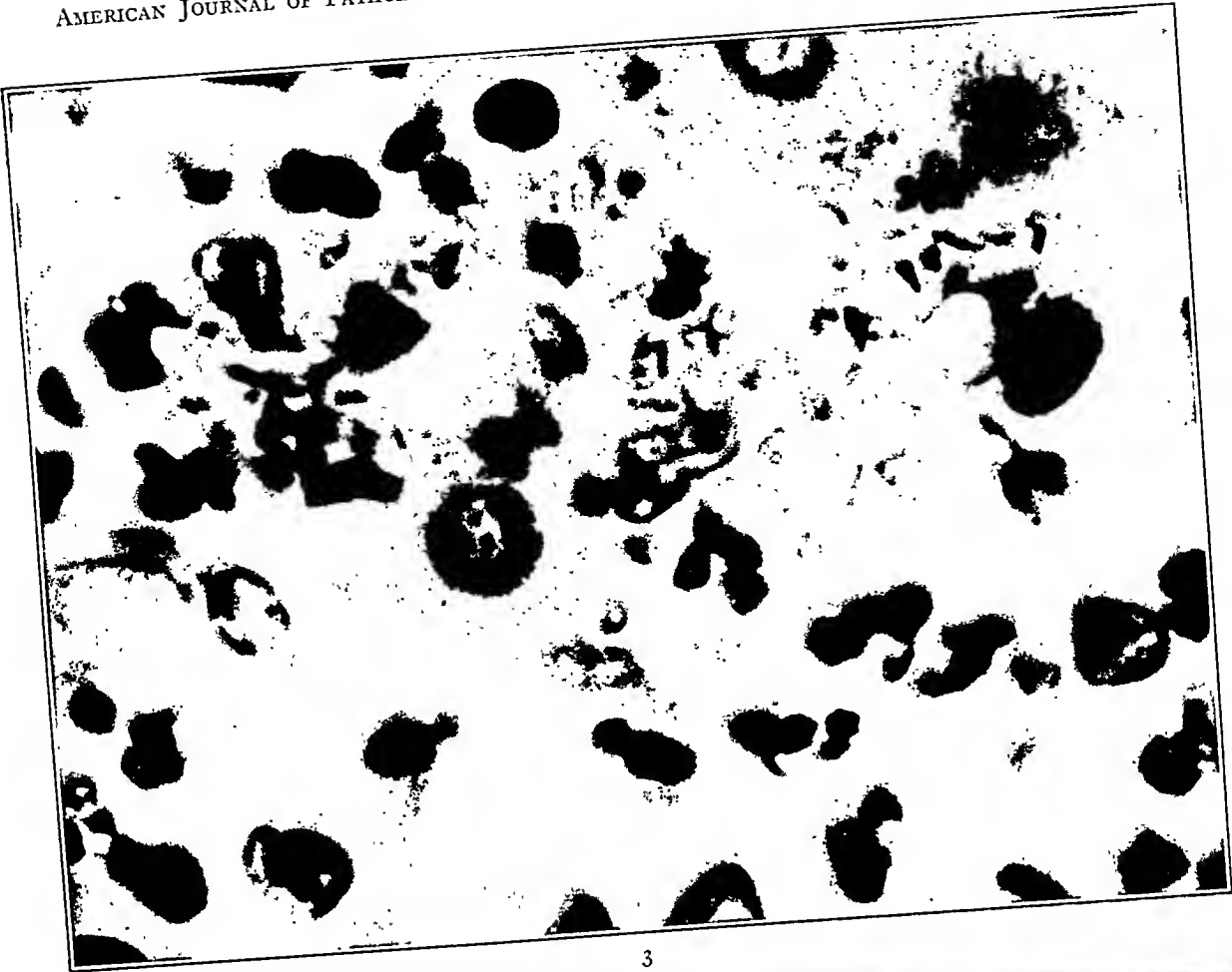


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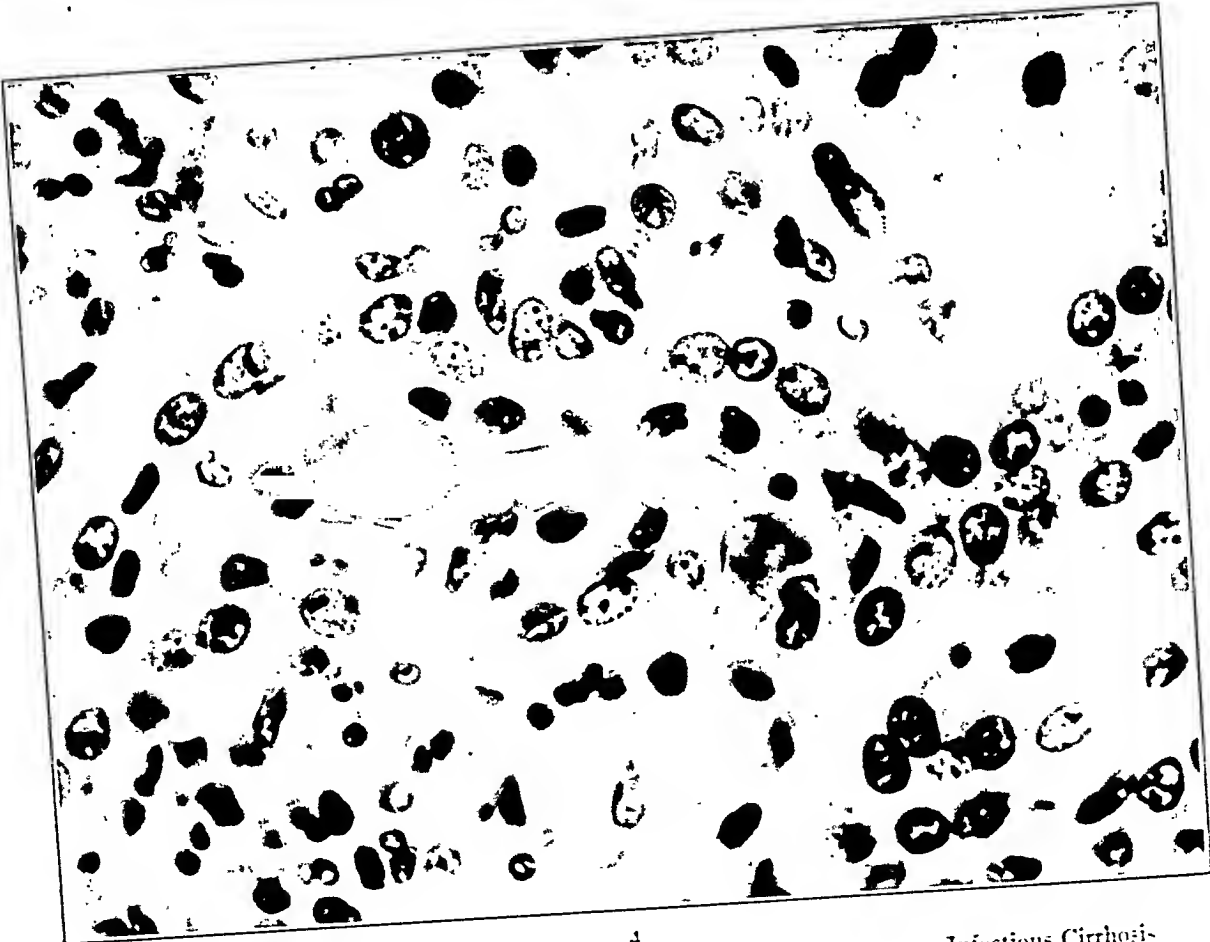
Infectious Cirrhosis

## PLATE 17

FIGS. 6, 7 and 8. Healed obstructive and infectious cirrhosis from a man 40 years of age. Weight of liver 2495 gm. Jaundice, esophageal varices. The lesion is very evenly distributed throughout the liver. The surface is granular. The photomicrograph shows that the lesion is situated around the portal vessels. The even distribution of the lesion, in contrast to that seen in Fig. 5, is probably due to the bile stasis which enabled the infecting agent to spread uniformly throughout all the dilated bile ducts.  $\times 50$ .



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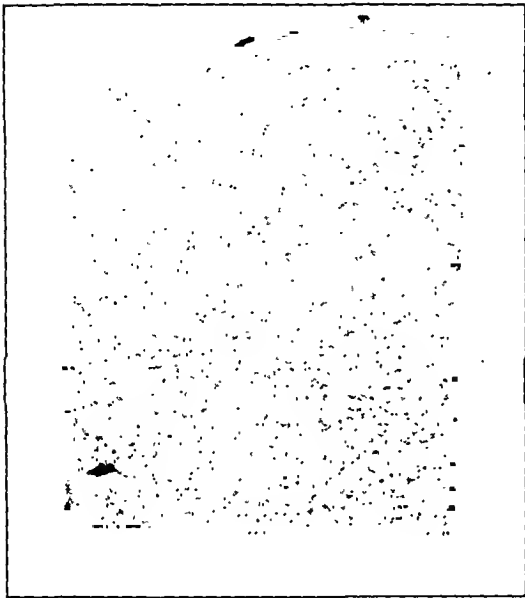


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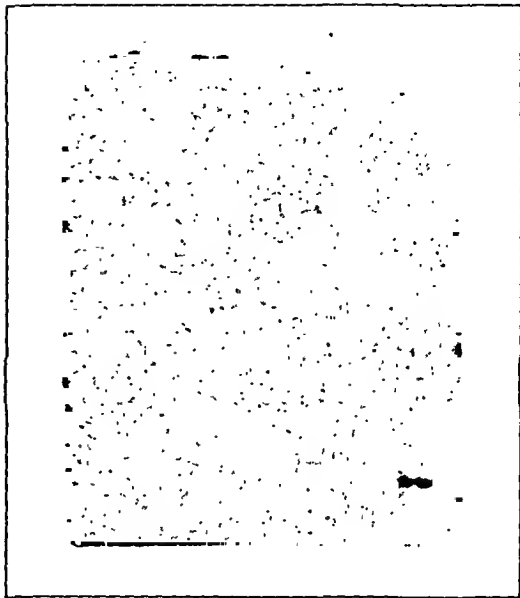
Infectious Cirrhosis



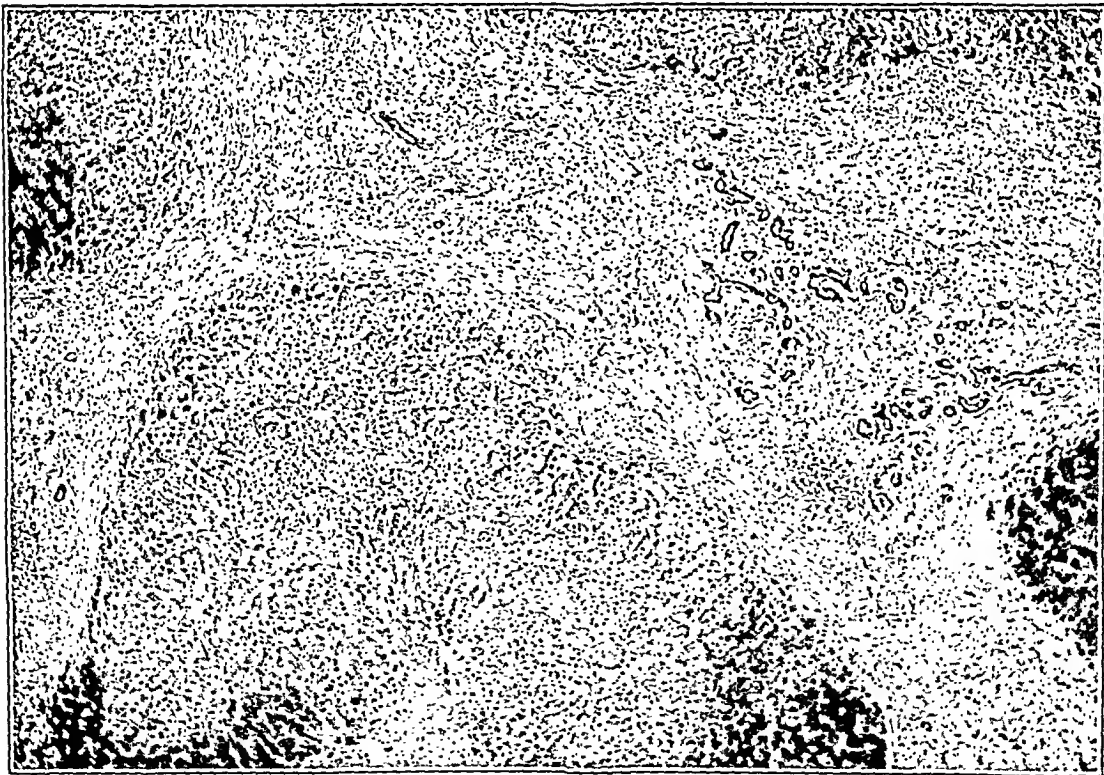




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arranged and clear, with small peripheral nuclei like fat cells. In another they are both diffuse and alveolar, very large, with central or peripheral nuclei and show a cytoplasm filled with small xanthoma-like droplets. There are many tumor giant cells. In neither of these cases was the material available for fat stains at the time the diagnosis of liposarcoma was suspected. In the third case, however, the biopsy was so suggestive that when the case finally came to autopsy, tissues were stained with Sudan III and the alveolar cells were found to be loaded with finer or coarser fat droplets. In ordinary osteogenic sarcoma the cells contain practically no fat. The author is very hesitant in advancing the notion that these tumors are liposarcomas and has withheld publication of these cases in the hope of gathering more conclusive material at some subsequent date. However, examples of this disease are rare and it was at last decided that these cases should be published to enable other pathologists to look for similar tumors. Brief summaries of the three cases follow.

### CASE REPORTS

CASE 1. G. P., male, aged 33 years, entered the Hospital July 26, 1926. His history prior to the onset of the present illness was uneventful. Four years ago (1922) a swelling appeared on the inner aspect of the third finger of the right hand. The finger was amputated at Bellevue Hospital, a diagnosis of sarcoma was made, and the patient remained free from further symptoms until eighteen months later. At that time there was slight swelling of the right hand and forearm which progressed steadily until admission to the Memorial Hospital. At the time of admission the forearm was diffusely swollen and radiographs revealed complete, or nearly complete destruction of the radius, slight destruction of the ulna and pathological fracture. Radiographs of the chest revealed no metastases. The arm was amputated.

*Gross Description of Tumor:* Arm amputated through lower humerus. The radius was replaced by a huge tumor mass (Fig. 1) fusiform in shape, 22 cm. long and 13 cm. in diameter, displacing the muscles but encapsulated by a distended periosteum. Of the bone, but the upper 2 cm. and the lower 1 cm. remained. The tumor seemed to have developed in the radius and to have replaced almost the entire bone. It was cystic in the center, 70 cc. of straw-colored fluid having been aspirated. About this central cavity the tumor mass was necrotic and this necrosis extended nearly out to the capsule. Especially along the lower end there were some soft, cellular, apparently growing areas. The main artery crossed over the tumor

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## PRIMARY LIPOSARCOMA OF BONE \*

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Out of the heterogeneous group of primary bone tumors have gradually emerged certain more or less defined clinical pathological entities — the benign giant cell tumors, the osteogenic sarcomas of varying structure, origin, course and prognosis, the endothelial myeloma of Ewing, primary angioendothelioma, various types of myeloma, erythroblastoma and possibly primary lymphosarcoma of bone. The only constituent of bone or bone marrow unrepresented in the tumor field seems to be the fat tissue which makes up such a large portion of quiescent marrow.†

In the extensive collection of bone tumors at the Memorial Hospital, opportunity has been afforded for the study of many varieties of bone tumors. From time to time the laboratory has been puzzled in properly classifying certain types of perithelial, alveolar or diffuse bone tumors. The tendency for a time was to regard such tumors as metastatic, and various organs, notably adrenal, kidney and thyroid, have been incriminated as the primary focus of disease. Nevertheless both clinical and autopsy evidence indicate that there exists a certain small group of pseudo-epithelial, spindle or polyhedral cell, diffuse or alveolar tumors of bone which are not metastatic, and there are reasons to believe that such tumors take their origin in the fat tissue of the marrow and are therefore liposarcomas. In one tumor which we have regarded as a liposarcoma the cells are diffusely

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† Nienhuis, J. H. (*Ztschr. f. Krebsforsch.*, 1925, 22, 434), describes bone involvement in liposarcoma, but in reviewing his case it is apparent that he regards the bone tumors as metastatic from a primary, or possibly multiple primary soft part tumor of fat tissue.

vealed pulmonary metastases. On June 2, 1926, since the chest metastases appeared stationary and since a pathological fracture of the tibia, present on admission, had failed to unite under treatment, an amputation was performed.

*Gross Description of Tumors:* The tumors were situated as described in the clinical summary. As in the preceding case, they apparently arose in the bone (Fig. 2), distending and pushing aside the soft part structures but remaining completely encapsulated. The upper tumor measured 9 cm. in diameter, the lower 14 cm. in length and 8 cm. in diameter. They were of a peculiar light yellow color, soft, but coherent. The lower femur, os calcis and small bones of the foot were the seat of numerous soft, hemorrhagic lesions grossly resembling angiomas, and the lesion was thought to be an angioendothelioma.

*Microscopic Examination:* The histology in this case is very bizarre. The tumor is made up in part of large, loosely arranged cells with relatively small, central or peripheral, deeply hyperchromatic nuclei (Fig. 5). In the cytoplasm are numerous xanthoma-like droplets which for the most part do not fill the entire cell. These peculiar cells do not line blood vascular channels. In other areas the tumor is comprised of small, closely packed spindle cells (Fig. 6). Sections from the above described vascular lesions of the marrow of other bones apart from the main tumor reveal small hemorrhagic foci of the fat tissue (Fig. 7). There is polymorphonuclear invasion of the fat tissue, some mucinous degeneration and the appearance of "young" fat cells. The process shows no neoplastic features but nevertheless appears to be rather significant in that this multiplicity of the vascular lesions of the other bones suggests one case of soft part liposarcoma observed during the past year. This tumor had developed in the soft tissues of the lower extremity, and in the amputated specimen in addition to the main tumor which was clearly liposarcomatous, there were peculiar fat changes all along the leg from ankle to knee. The fat lobules were unusually small, the cells were small and there was a diffuse nodular injection although the individual fat nodules showed no neoplastic tendencies.

*Comment:* In this second bone tumor the diagnosis of liposarcoma is based upon the peculiar gross appearance, alveolar character, xanthoma-like droplets in the individual cells, absence of evidence of origin from endothelium, lack of resemblance to any known variety of primary bone tumor and the clinical course, *i.e.*, the ab-

with no definite entrance into it. The metacarpal bone of the amputated phalanx was normal.

*Microscopic Examination:* The sections show a very peculiar tumor made up in part of small hyperchromatic, spindle and polyhedral cells. Much of the material was unsuited for study on account of necrosis. However, in some regions (Fig. 3) the tumor is comprised of large clear cells with small, generally peripheral and at times flattened, hyperchromatic nuclei resembling fat tissue. The diagnosis of liposarcoma is based partly on these areas and partly on the clinical course.

*Comment:* The tumor is certainly not the usual bone tumor. It began apparently in a phalanx where we do not see osteogenic sarcoma. It did not recur locally but developed either by venous extension or entirely *de novo* in another bone of the same extremity, grew to enormous size but failed to metastasize. It did not behave like any osteogenic sarcoma or endothelial myeloma we have ever seen. It was not metastatic since it is quite inconceivable that the tumor could have grown to such bulk in two different bones and over so long a period, with the primary tumor, if such there were, remaining symptomless. Furthermore, the patient is still well. In other words we have a bone tumor arising in a peculiar location, showing a picture histologically suggestive of a fat tumor, no evidence of any other primary lesion and a very unusual clinical course. We believe the diagnosis of liposarcoma is justified in this case.

CASE 2. The second case was a male, aged 28 years. He entered the Memorial Hospital August 29, 1924. His illness was of approximately one year's duration, with the initial symptom pain in the calf of the left leg. A physician, apparently noting some swelling, had attempted to aspirate the lesion. Nothing was obtained and the pain increased. The patient entered a hospital in Sayville, Long Island, where deep in the left calf a smooth mass some 5 cm. in diameter was noted. The mass was thought to be in the soft tissues. It did not pulsate but yielded a bruit on auscultation. A popliteal aneurysm was considered in the differential diagnosis. X-rays were reported negative. The patient was operated upon and the surgeon removed what he considered to be the whole tumor. He reported it encapsulated but adherent over the posterior surface of the head of the fibula, that is, closely applied to bone but not arising from bone. A pathologist rendered the report "alveolar sarcoma." By July of 1924 there was a definite recurrence. On admission to the Memorial Hospital the head of the fibula was the site of a globular, pulsating, tense tumor mass 5 cm. in diameter. Over the anterior surface of the left tibia at the junction of its middle and lower thirds was a second tumor producing diffuse swelling, slight edema and very marked tenderness, covering an area 8 by 5 cm. Radiographs of the chest re-

medullary cavity. On one aspect the cortex was not involved, but on the other the neoplastic tissue eroded bone and extended nearly to the surface. The material again was soft, rather pulpy and brick red in color and was sharply demarcated from the surrounding marrow which was very fatty.

*Microscopic Examination:* Sections reveal a tumor suggesting an alveolar carcinoma. The alveolar structure is well developed. The cells are irregular in size, rather polyhedral in shape; some are opaque, others coarsely or finely vasculated. This alveolar appearance is maintained in all of the metastases. The stroma is fibrous and in places fairly abundant. Sudan III stains show that the tumor cells are loaded with fat in coarser or finer droplets (Fig. 4). Many of the cells approach the large polyhedral cells observed in the preceding case.

*Comment:* The diagnosis of liposarcoma in this instance is based on the absence of primary tumor in a thoroughly autopsied case, the abundant fat, and the lack of resemblance to any other known tumor.

### SUMMARY AND CONCLUSIONS

In reporting these three cases of bone tumor as liposarcomas we are fully cognizant of the fact that we are in doubtful territory. In no case has it been possible to trace the actual origin of the tumor to fat cells, although in one instance the inflammatory fat changes in the marrow apart from the tumor were very suggestive. The conclusions drawn rest entirely on circumstantial data: (1) the resemblance of the tumor cells to fat cells, the presence of large fat droplets in one case, the general resemblance to fat cells in another although no specific fat stains are available, and the xanthomatous droplets in the second case; (2) the peculiar clinical course, namely, a suggestion at least of multiplicity of bone lesions in all three instances which recalls the behavior of certain of the liposarcomas we ourselves have observed in the soft parts; (3) lack of evidence of primary epithelial origin, and (4) an appearance inconsistent with primary bone tumors of known types.

That the disease is more or less of a clinical entity may be surmised from the fact that all three of the tumors were either multiple in bone or else showed a pronounced tendency to metastasize to other bones, and that of the two cases treated by irradiation both

sence of evidence of any primary epithelial tumor over a period of six and one-half years. The clinical course was peculiar in that the pulmonary metastases remained well under control by radiation up to the time of death, more than five years after admission. Death was apparently due to intracranial metastases.

CASE 3. G. H., male, aged 28 years, entered the Memorial Hospital on April 4, 1930. It was almost impossible on account of the patient's nationality to determine the sequence of events in the course of his illness. A short time prior to his admission at Memorial he had attended another hospital for diagnosis, and on the basis of radiographs his ailment was thought to be due to multiple myeloma involving the right femur, the left parietal bone, right seventh rib and midthoracic vertebrae. To establish the diagnosis an exploration of the femoral tumor which had produced a large soft part swelling was performed. The mass was soft, pinkish and very vascular. A portion of the tumor was excised for diagnosis but no positive opinion could be rendered. Our own diagnosis on the slides from this operation was inconclusive. The possibilities were in order (1) primary alveolar liposarcoma of bone, (2) angioendothelioma, (3) metastatic thyroid or adrenal carcinoma. In this report we called attention to the resemblance between this case and Case 2 of this series. Under X-ray treatment the tumors proved very radiosensitive. Nevertheless the disease was far advanced on admission. Evidence of spinal cord compression was apparent. The patient was incontinent and died eleven days after admission. Complete autopsy was performed, including the neck organs, and no primary tumor could be discovered.

*Postmortem Examination:* There was a diffuse subcutaneous swelling above and behind the left ear as one frequently sees overlying destructive tumors of the cranial bones. Beneath it an area of bone destruction in the parietotemporal region could be palpated. The tumor was soft, reddish, pale brick-colored, vascular, granular and sharply demarcated from the surrounding bone.

The left lung and pleura were studded with very small metastatic nodules, pinkish and vascular. There was a soft, pinkish mass, ovoid in shape, tense and elastic, measuring about 4 by 4 by 8 cm. overlying the sixth, seventh and eighth ribs adjacent to the vertebral column on the right. On section this mass was soft, brick red in color and pulpy. It extended into the vertebrae and a finger introduced into the tumor could be passed directly down to the spinal cord. Aside from the tumor of the femur no other lesions were found. The latter tumor was situated at the junction of the upper and middle thirds of the femur. No soft part mass was found (the tumor had regressed under radiation). The outer cortex of the bone was roughened but otherwise normal. The tumor occupied the



## DESCRIPTION OF PLATES

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### PLATE 18

FIG. 1. Amputated arm, Case 1. Middle finger previously amputated at another hospital. Destructive, semicystic tumor involving nearly the entire radius.

FIG. 2. Tibia, Case 2. Shows the lower tumor only.

proved radiosensitive. The bony tumors regressed very markedly in the one and the pulmonary metastases were long held in check in the other. This radiosensitivity is quite inconsistent with either a primary bone tumor of the osteogenic variety which even remotely approaches our liposarcomas in structure, or with a metastatic adenocarcinoma, but it is not inconsistent with certain liposarcomas we have observed in the soft tissues. It is of further interest from the clinical standpoint that whereas in osteogenic sarcoma cranial metastases are rather uncommon, in two of the three tumors we have regarded as liposarcoma cranial involvement has occurred.

PLATE 19

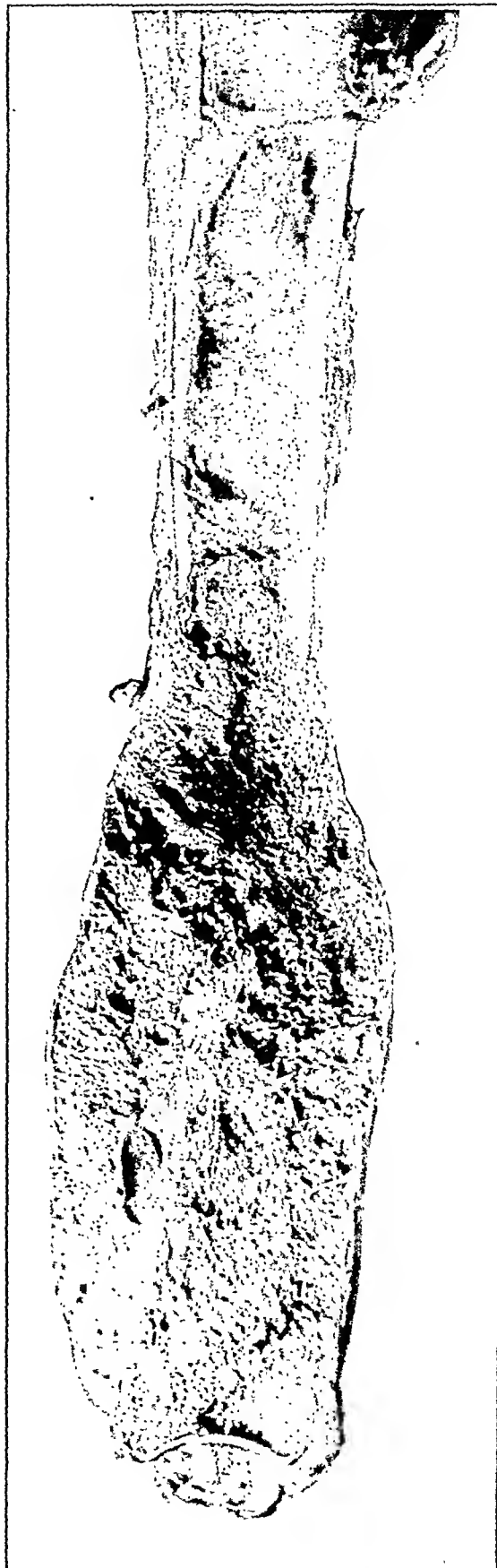
FIG. 3. Case 1. Diffuse tumor mass in which the cells resemble small fat cells.

FIG. 4. Case 3. Tumor cells in alveolar arrangement. Marked variations in size and shape. Much Sudan III stainable fat.



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Stewart

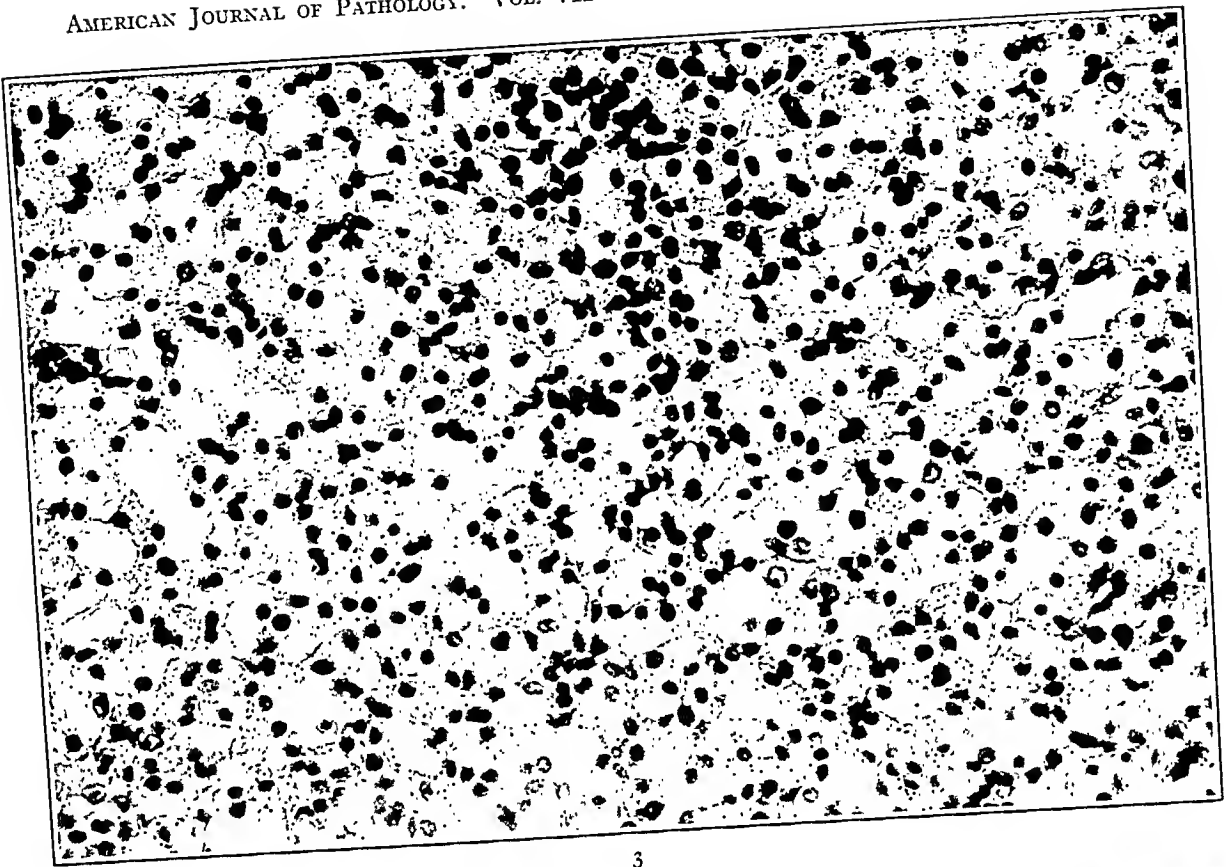


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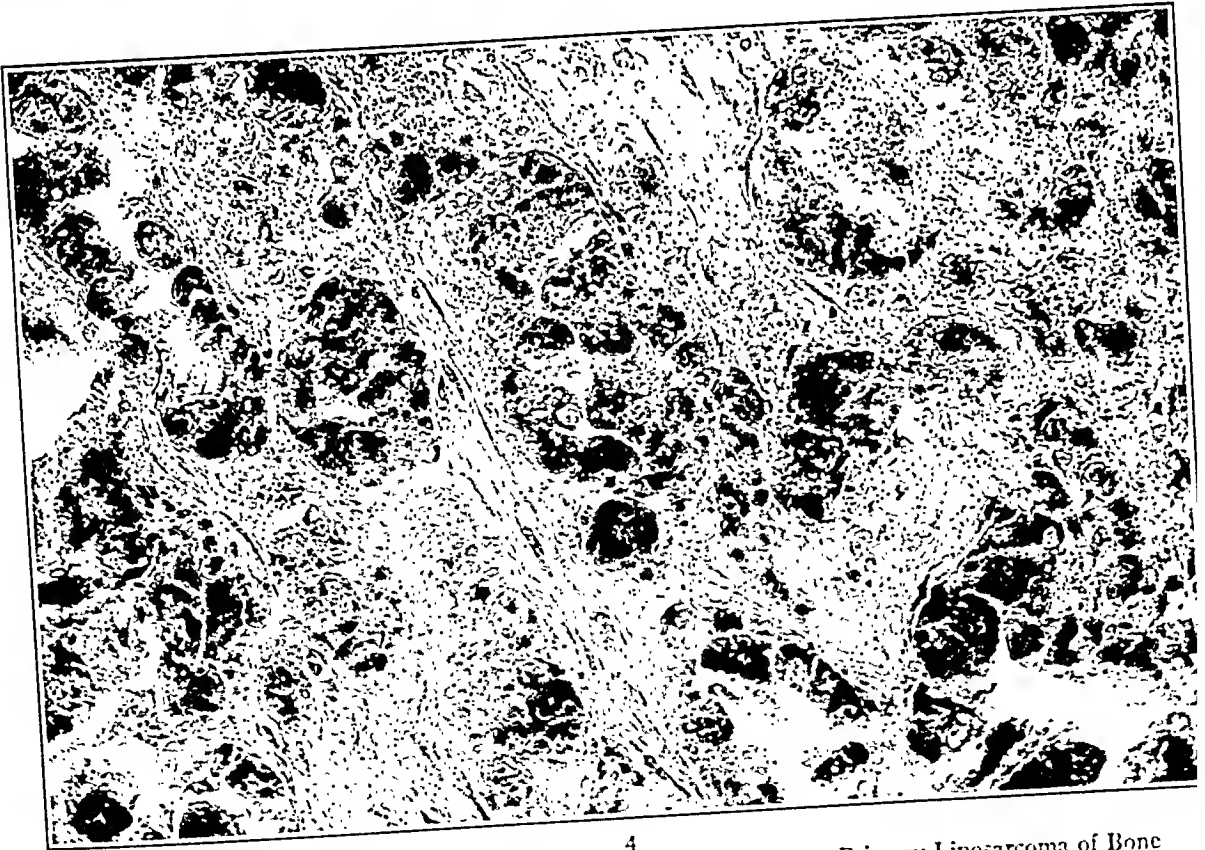
Primary Liposarcoma of Bone

PLATE 20

- FIG. 5. Case 2. Masses of large polyhedral cells in vaguely alveolar formation. Marked variation in size and shape. Nuclei often peripheral. Numerous xanthoma-like droplets.
- FIG. 6. Case 2. Another area showing the tumor comprised of numerous small spindle cells.
- FIG. 7. Case 2. Cells taken from the hemorrhagic areas apart from the tumor. These show the morphology of young fat cells. There is mucinous degeneration of the marrow and invasion of some of the fat cells by polymorphonuclear leukocytes.



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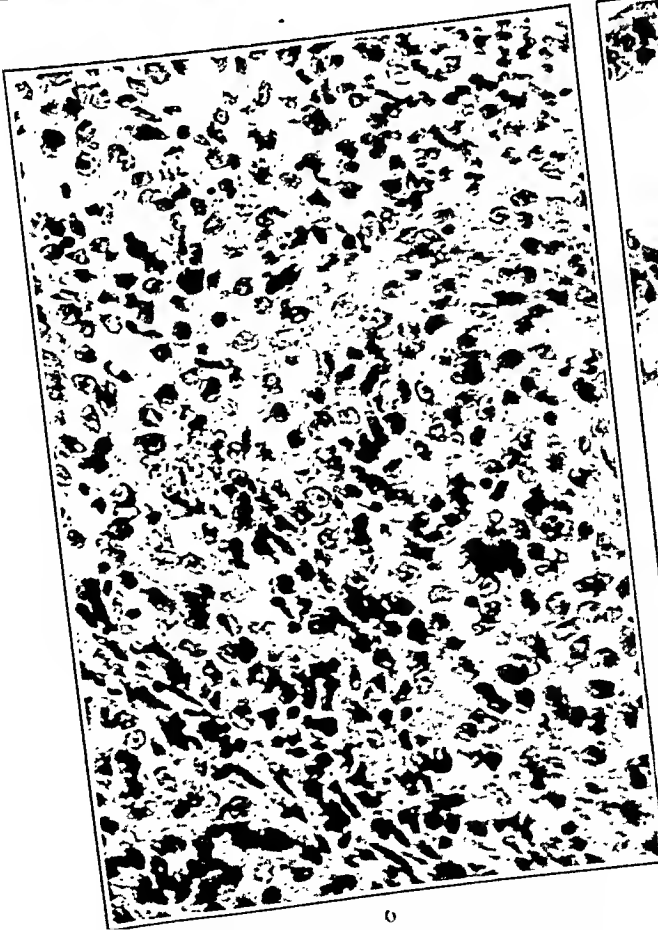
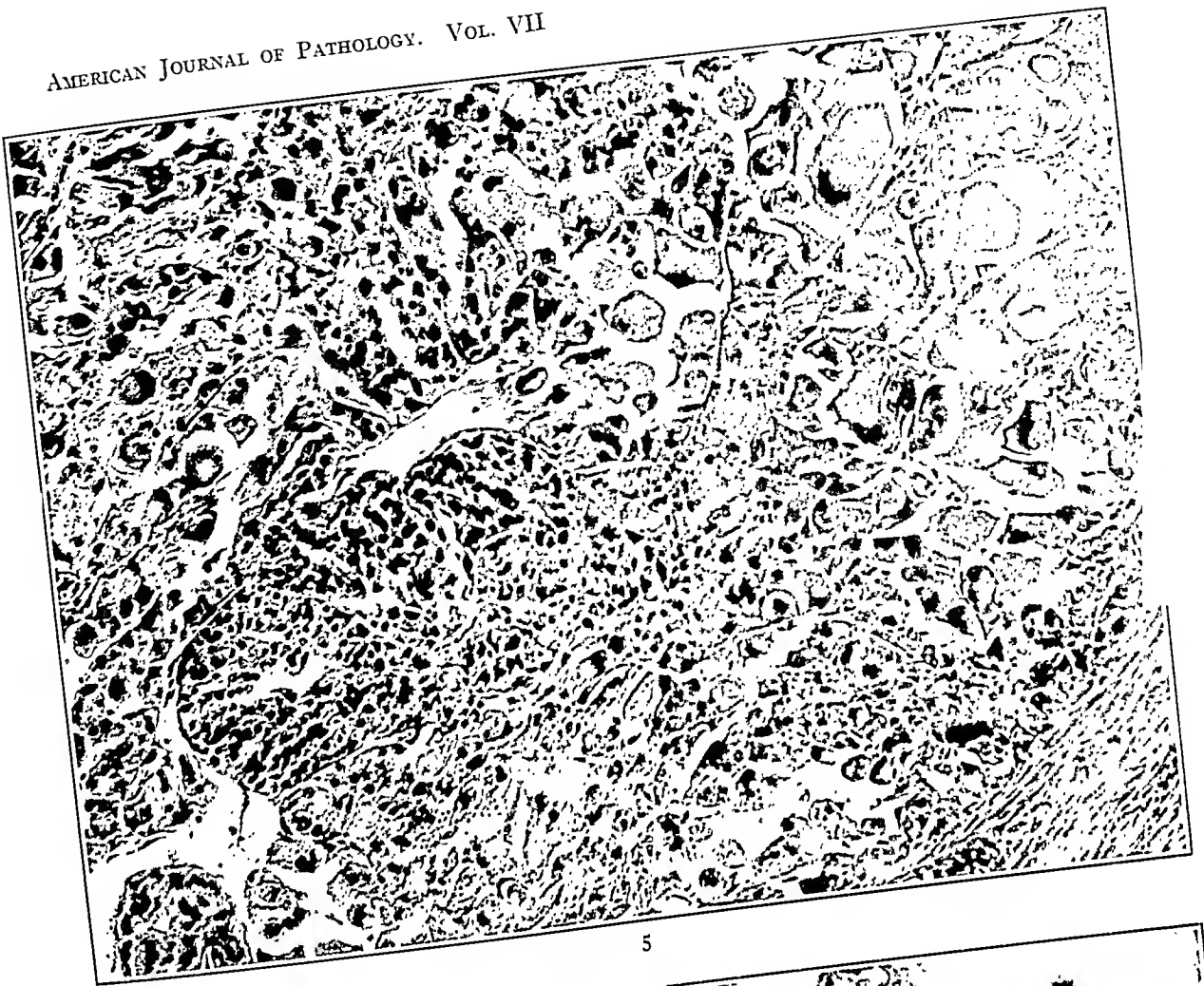


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Primary Liposarcoma of Bone

Stewart





Primary Liposarcoma of Bone



Atlantic states, the disease is endemic. On the whole, it seems more probable that Brill's cases were also of native origin.

The mortality from endemic typhus in this country is low and material for pathological study is therefore difficult to obtain. In 1911 Brill<sup>4</sup> described the autopsy findings of one case, and of two other probable cases. The pathology of typhus was not understood at that time and the brain (the most important organ from a diagnostic point of view) was not studied microscopically in any of these three cases.

Autopsy material from the fatal case to be reported here was obtained under ideal conditions, and a comparison of the pathology (in the human) of this American strain of typhus with that of European typhus was made possible. The pathology of European typhus has been studied in great detail by Wolbach, Todd and Palfrey<sup>5</sup> and the material on which their studies were based was available for purposes of comparison.

## REPORT OF CASE

*Clinical History:* The patient was a white man, 53 years old, who lived about a mile west of Charlottesville, Virginia, in an isolated farm house. He worked as a dairyman at a nearby farm. For a month preceding the onset of his illness he had not been away from his home except for an occasional trip to Charlottesville. There was no history of contact with a preceding case and no known contact with any stranger of foreign origin. The man was the father of a large family and stayed at home most of the time. There were no secondary cases in the family or neighborhood, or in the hospital to which he was admitted without special precautions. He gave no history of lice or of other insect bites.

The onset of his illness was rather abrupt. On June 12 he developed a severe headache, joint and muscle pains, general malaise, nausea without vomiting, and fever. He was admitted to the hospital on the seventh day of his illness. At the time of his admission he appeared to be very toxic and it was impossible to get a coherent story from him. On the second day after admission a rash was noted over the trunk. This spread rapidly and soon involved the whole body. It was distinctly maculopapular in character and in the later stages became petechial. The eruption was so intense that it was plainly visible across the room. His temperature continued elevated, his pulse rapid, and his mental dullness deepened into coma. His condition became worse, respirations increased to a marked hyperpnea and he died on the eleventh day. On the final day of his illness the scrotum was examined and there was no apparent involvement of the testes.

Laboratory findings were as follows: The blood pressure was 170/100, hemoglobin 78 per cent, red blood count 5,400,000, white blood count 5,400, differential count within normal limits. The agglutination of *Proteus* X19 was negative on admission. The Wassermann reaction was negative. Lumbar puncture was

# PATHOLOGICAL STUDY OF A CASE OF ENDEMIC TYPHUS IN VIRGINIA WITH DEMONSTRATION OF RICKETTSIA \*

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## INTRODUCTION

Endemic typhus is definitely established as a disease entity in the United States, and the number of cases occurring in recent years (Maxcy <sup>1</sup>) has been sufficiently large to make the condition of considerable medical interest.

When Brill<sup>2</sup> in 1910 reported 120 cases of typhus in New York, the nature of the condition was so uncertain that he preferred not to give it a definite name. In 1912, Anderson and Goldberger<sup>3</sup> infected monkeys with the virus of this "unknown disease" and showed by cross immunity tests that it was in all probability essentially identical with Mexican typhus, but this evidence was not universally accepted.

In 1923, when Maxcy began his studies of the problem, he was aided greatly by the Weil-Felix reaction, and also by the fact that the experimental pathology of the disease in the guinea pig had been worked out. He was able repeatedly to establish the disease in guinea pigs and demonstrated the complete identity of his strains with Mooser's strain of Mexican typhus, and the essential identity (cross immunity) with two strains of European typhus. The differences between the Old World and New World strains in the guinea pig will be referred to later.

Maxcy has presented strong epidemiological evidence against the louse transmission of the disease in this country. His studies suggest that some other insect vector will probably be found, and there is considerable reason for believing that there is a natural reservoir for the disease in rodents, possibly rats or mice.

The cases reported by Brill were considered by many students of the problem to represent an importation of the disease from Europe, but the more recent work has shown that, at least in the south

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capillaries of the heart wall by a process of endothelial proliferation, followed by a perivascular accumulation of macrophages and other cells. These lesions in the myocardium were entirely similar to those described by Wolbach, Todd and Palfrey in their European cases. There was moderate diffuse fibrosis of the myocardium, clearly an old process and not related to the acute illness.

The diagnosis of bronchopneumonia of the right lower lobe was confirmed histologically. Many of the alveoli contained only red blood cells and serum, and the infection had the appearance of a terminal one. In the uninvolved regions no lesions characteristic of typhus were present.

The spleen was moderately congested. The trabeculae and follicles were essentially normal in appearance and the pulp showed a predominance of mononuclear cells. The reticulo-endothelial cells were somewhat prominent. Occasional small collections of large mononuclear cells somewhat suggestive of typhus "nodes" were present and the venous sinuses in several instances appeared to be occluded by proliferation of their lining cells, but the structure of the spleen makes the recognition of proliferative vascular lesions almost impossible.

The liver showed accumulations of mononuclear cells in the portal areas and slight proliferation of the Kupffer cells, but the changes were such as occur frequently in other conditions.

The testes showed somewhat reduced spermatogenic activity which was not considered remarkable in view of the age of the patient. In the tunica albuginea, one group of adjacent small capillaries showed involvement of the type characteristic of typhus infection. There was no inflammatory reaction on the surface of the tunica.

The kidneys, pancreas and aorta showed no specific typhus lesions. Unfortunately no sections were made from the infarct in the kidney.

The most marked involvement was found in the brain. Of thirty-six sections studied, representing various regions in the cerebral cortex, basal ganglia, pons, cerebellum and brain stem, not one failed to show characteristic and even diagnostic lesions. These typhus lesions have been so carefully studied and described elsewhere that it is unnecessary to go into great detail. The earliest lesion is represented by small vessels which show prominence of the endothelium,

done on the day of admission and the findings were those of a normal fluid. The urine showed an occasional finely granular cast but no blood. The blood urea nitrogen was 112 mg. per 100 cc. Blood taken on the eleventh day after admission agglutinated *Proteus* X19 in a dilution 1:2560.

The clinical diagnosis was typhus fever.

Autopsy was performed nine hours after death by Dr. E. S. Groseclose.

### POSTMORTEM EXAMINATION

There was moderate emaciation, and the characteristic fine petechial eruption was present on the skin, especially on the hands, feet, buttocks and lower back.

The right lung showed dark red pneumonic consolidation of its lower lobe. Pleural scars were present at the right apex.

The right kidney contained an old white infarct on its lateral border measuring 2 by 1 cm. on cut surface. Otherwise the kidneys appeared negative.

The brain was markedly congested, rather soft and pinkish in color on section.

The peritoneal, pleural and pericardial cavities, heart, spleen, pancreas, liver, gall-bladder, gastro-intestinal tract, aorta and testes were essentially negative on gross examination.

The anatomical diagnoses were: early bronchopneumonia of the lower lobe of the right lung, healed tuberculosis of the apex of the right lung, old infarct of the right kidney and petechial eruption on the skin.

### MICROSCOPIC EXAMINATION

The heart showed numerous lesions. Many of the precapillaries showed definite intimal proliferation and thrombus formation with perivascular accumulation of macrophages and lymphocytes. The thrombi were composed chiefly of endothelial cells and platelets, but in one instance eight or ten polymorphonuclears were present. A second type of lesion consisted of fusiform collections of cells between spread-apart, but not obviously damaged, muscle fibers. These cells included macrophages, endothelial cells, lymphocytes, plasma cells, mast cells and polymorphonuclears, their frequency being in the order mentioned. At first glance these lesions resembled focal necroses, but the muscle fibers did not appear damaged. On more careful study it was obvious that the great majority (and, by deduction, probably all) of these lesions had originated in the minute

were brought out so clearly in the cytoplasm of the proliferating endothelial cells that it is felt that the method used is possibly the method of choice for brain tissue. For this reason, the treatment which the tissues received will be given in detail.

The brain was fixed *in toto* in formaldehyde of the usual strength (4 per cent solution). One month later blocks 4 to 8 mm. in thickness were cut from various parts of the brain, placed in bottles of fresh formaldehyde of the same strength and sent to Boston. These blocks were then further cut to a thickness of 2 to 3 mm., washed in running water for 24 hours and then fixed for 36 hours in Regaud's fluid.\*

After fixation the tissues were washed for 24 hours, dehydrated in graded alcohols, cleared in cedarwood oil and embedded in paraffin in the usual way. Sections were cut as thin as possible, and it is believed that this is a fairly important part of the technique.

The sections were stained overnight in Giemsa solution (2.5 cc. of concentrated solution, 2.5 cc. of methyl alcohol, 100 cc. of distilled water and 5 drops of 0.5 per cent sodium carbonate), changing the staining fluid twice during the first three hours.

The sections were stained a uniform deep blue and it was impossible to get satisfactory differentiation with colophonium or acetic acid, since both tend to decolorize the rickettsia before they produce color changes. Exposure to strong sunlight slowly differentiates the sections without much fading. The best results were obtained by carrying the differentiation to the point at which the red blood cells have turned from green to pink. The rickettsia at this stage stand out as deeply stained bluish purple structures in the faint blue cytoplasm of the endothelial cells. The majority of the organisms

\* Regaud's fluid was first used (in a modified form) for the demonstration of rickettsia by Rosenberger.<sup>6</sup> Later it was used by Cowdry<sup>7</sup> in studying the rickettsia of heartwater. He gives the formula as 4 parts of 3 per cent potassium bichromate and 1 part of formaldehyde (40 per cent). The formula used here is that given in McClung's Handbook of Microscopical Technic:

Potassium bichromate.....	25 gm.
Sodium sulphate.....	10 gm.
Distilled Water.....	1000 cc.

Before using, add 20 cc. of 40 per cent (actual) formaldehyde to every 100 cc. of fixative.

For both human and guinea pig tissues, this has been found to be far superior to all other fixatives tested, and rickettsia are stained as sharply and as deeply as ordinary bacteria.

and occasional mitoses of the endothelial cells *in situ*. The lumen becomes obliterated by the proliferated endothelial cells and by the platelets and fibrin which collect as a result of damage to the lining of the vessel. Macrophages and cells of the lymphocytic series collect in the perivascular space, neuroglia eventually proliferates and the late lesion is a mass of closely packed cells of various types so that the vascular origin of the lesion eventually becomes obscured. Many vessels showed perivascular accumulations of mononuclear cells, some of which were in mitosis, without noticeable endothelial proliferation. In serial sections, however, the typical picture of endothelial proliferation was often found at some point along the course of such vessels. Large numbers of enormous phagocytic cells containing hemosiderin and chromatin inclusions were often seen in the perivascular spaces of the larger vessels, but the intima of these larger vessels was apparently normal.

Many of the thrombi contained numerous polymorphonuclears and pyknotic nuclear fragments. This feature was more marked than in the usual case of European typhus, and the lesions in the case reported here seemed unusually numerous and acute. Often there was a zone of vacuolization of the brain substance about these acutely thrombosed vessels, similar to that seen in an ordinary brain infarct. Another type of lesion seen in sections representing the internal capsule in this case was a small circumscribed area of hyaline degeneration of the white matter without cellular reaction. This appearance was apparently produced by agglomeration and fusion of small groups of damaged nerve fibers and probably represents paths of fiber degeneration, secondary to destruction of ganglion cells by involvement in the larger vascular lesions, although it may be of arteriosclerotic origin and unrelated to the acute illness.

Sections of skin showed typical vascular lesions which need not be described since they were similar to those in the heart and brain. Mast cells were a prominent feature of these lesions as in all typhus skin lesions.

#### THE DEMONSTRATION OF RICKETTSIA

Because of the fact that the brain had been fixed in formalin (which is not generally believed to be satisfactory for this purpose) it was thought that the demonstration of rickettsia would be a difficult task. The organisms were found relatively easily, however, and

Palfrey. The rickettsia in the lesions also correspond morphologically to those described by these workers. The study has therefore confirmed both the essential similarity of American and European typhus and the etiological relationship of *Rickettsia Prowazeki* to typhus, although neither of these facts stood in serious need of confirmation.

The clinical differences between European and American typhus in the guinea pig are now quite well understood. Male guinea pigs inoculated intraperitoneally with American typhus virus react by a marked inflammation of the tunica vaginalis, and this reaction almost completely prevents the occurrence of the vascular lesions in the brain. For a time this fact caused confusion, in that it suggested a similarity of the disease to Rocky Mountain spotted fever, but the similarity is only apparent, since in the latter disease the reaction is primarily vascular and its occurrence is independent of the route of inoculation, while in typhus the reaction is chiefly in the lining cells of the tunica vaginalis and occurs only after intraperitoneal inoculation (Pinkerton<sup>8</sup>). When inoculation with American typhus material is done subcutaneously there is no reaction in the tunica, and brain lesions are frequently as numerous as in the European strain.

The absence of any inflammatory reaction in the tunica in the case reported here confirms the opinion already expressed that the tunica reaction in experimental infection does not represent a localization of the virus after gaining entrance to the blood stream, but is rather a reaction at the portal of entry when the artificial method of intraperitoneal inoculation is employed. The presence of numerous brain lesions in the reported case is what one would expect in view of the fact that natural inoculation in the human being is probably subcutaneous or intracutaneous and with relatively small numbers of rickettsia.

#### SUMMARY AND CONCLUSIONS

1. Study of a fatal case of endemic typhus in Virginia has established the complete pathological identity of the disease with European typhus.
2. Characteristic brain lesions were fully as numerous as in the average case of European typhus, and many of these lesions were unusually acute.
3. Involvement of the tunica vaginalis and scrotum was no more marked than in European typhus.

appear as definite rods, occasionally paired and rarely standing on end so that they resemble cocci. Definite coccoid forms were not seen, however.

With further exposure to sunlight, the organisms change to a light red color and then slowly fade. They may be restained and re-differentiated without loss of clarity. The apparent size of the organisms is greatly exaggerated when they are deeply stained, but as they differentiate and fade their true size becomes evident.

The organisms were found invariably and exclusively within the cytoplasm of the endothelial cells *in situ*, and were never definitely seen in the perivascular macrophages. They were not seen in neuroglia cells, ganglion cells, the cells which cover the meninges or those which line the ventricles. Many of the macrophages, especially in the later lesions, develop granular cytoplasm, but the granules cannot be recognized as organisms. In the very late lesions the entire background becomes a mass of minute blue-staining granules, often rod-shaped but never uniform in size and shape. Among these structures, purple-staining rickettsia in groups of 3 to 6 were occasionally recognized, but the majority of the granular structures were believed to be derived from broken up neuroglia fibrils. A similar appearance is often seen in the brain lesions of experimental typhus in the guinea pig, and a large number of sections have been prepared without ever bringing out these structures as definite organisms.

In several vessels as many as six endothelial cells were definitely observed to contain rickettsia. In many of the sections, by selecting under low power the earliest and most acute vascular lesions, it was possible to find rickettsia after a few minutes' search, but the majority of the lesions contained no demonstrable organisms. When found in a given lesion it was nearly always possible to find them in serial sections of that lesion mounted on the same slide.

The organisms were never very numerous, ranging from two or three up to eighteen or twenty in a single cell. They never packed the cytoplasm of the cells as they do in the gut of the louse and scrotal sac of the guinea pig.

#### COMMENT

The pathological changes in this case do not differ in any way from those described in the European series by Wolbach, Todd and



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## DESCRIPTION OF PLATES

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### PLATE 21

FIG. 1. Low power view of vascular lesions in brain stem.  $\times 80$ .

FIG. 2. A fairly late vascular lesion in the cerebral cortex. A suggestion of a central blood vessel is still present.  $\times 700$ .

4. *Rickettsia Prowazeki* were easily and clearly demonstrated in the endothelial cells of the vascular lesions in the brain, but were not found in macrophages or neuroglial cells.

5. A technique is described for the demonstration of rickettsia in formalin-fixed brain material.

6. The demonstration of *Rickettsia Prowazeki* in this material is to be added to the already overwhelming evidence in favor of their etiological relationship to typhus fever.

NOTE: While this paper was in press our attention was called to the recent work of Badger, Dyer and Rumreich.<sup>9</sup> These workers have shown that two closely allied diseases occur in southeastern United States. In addition to endemic typhus, as described by Maxcy, they report the occurrence of a disease which they regard as more closely allied to Rocky Mountain spotted fever. We have considered the possibility that the case reported here belongs to the group of cases which these workers describe. In view of the fact that brain lesions have not been described in spotted fever, either in human or animal tissues, and because of the complete pathological identity of our case with European typhus, it is impossible to believe that it belongs in the spotted fever group. It is, of course, unfortunate that animal inoculation was not done from this particular case.

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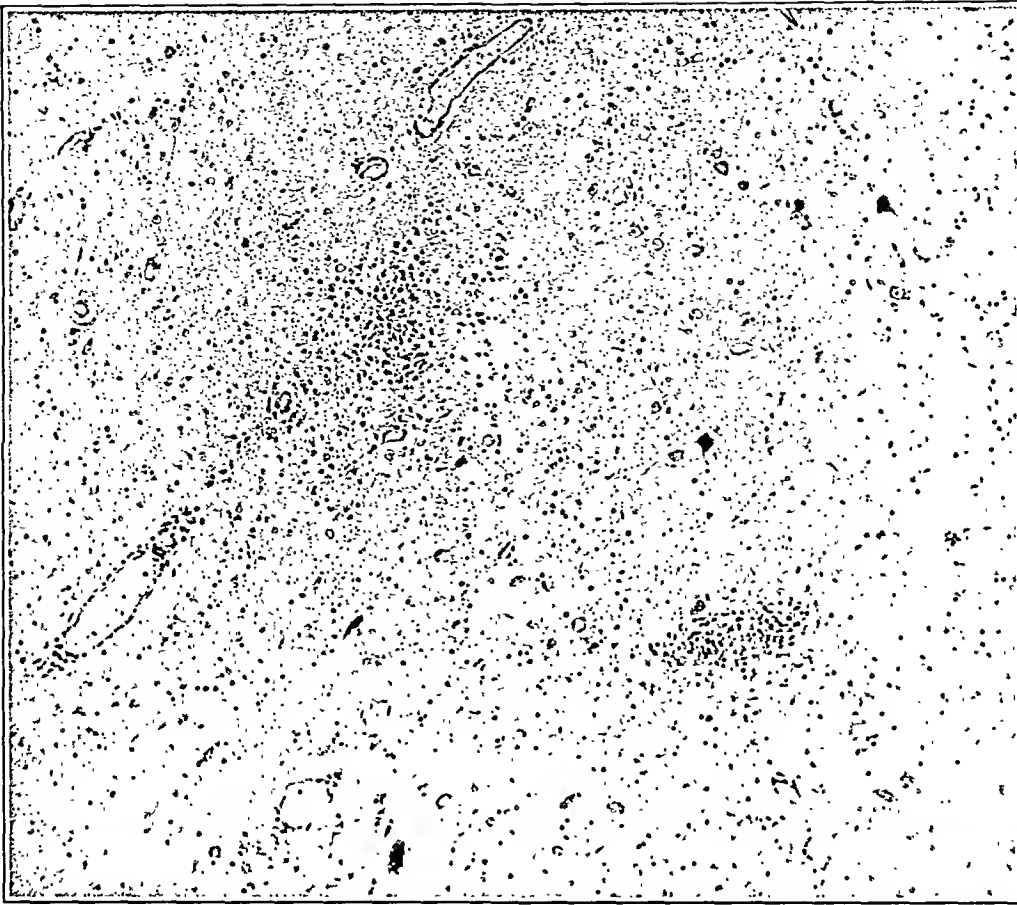
PLATE 22

FIG. 3. An acute vascular lesion in the cerebral cortex, showing early thrombus formation and perivascular infiltration. The arrows within the lumen point to endothelial cells containing rickettsia.  $\times 1500$ .

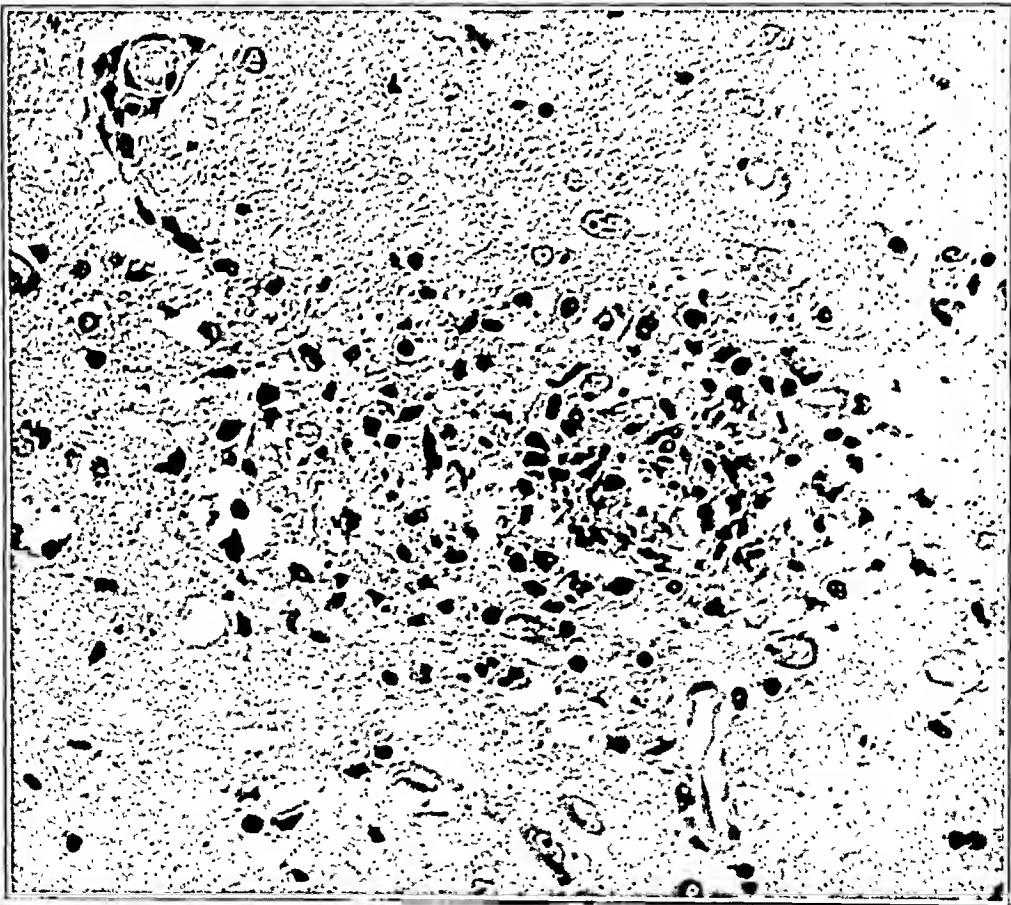
FIG. 4. An early lesion in the cerebral cortex showing rickettsia in upper endothelial cells.  $\times 1500$ .

FIG. 5. Same cell as that shown in Fig. 4, focused at a different level.  $\times 1500$ .

FIG. 6. Another group of rickettsia in same vessel as those in Figs. 4 and 5. One definite diplobacillus in a vertical position.  $\times 1500$ .

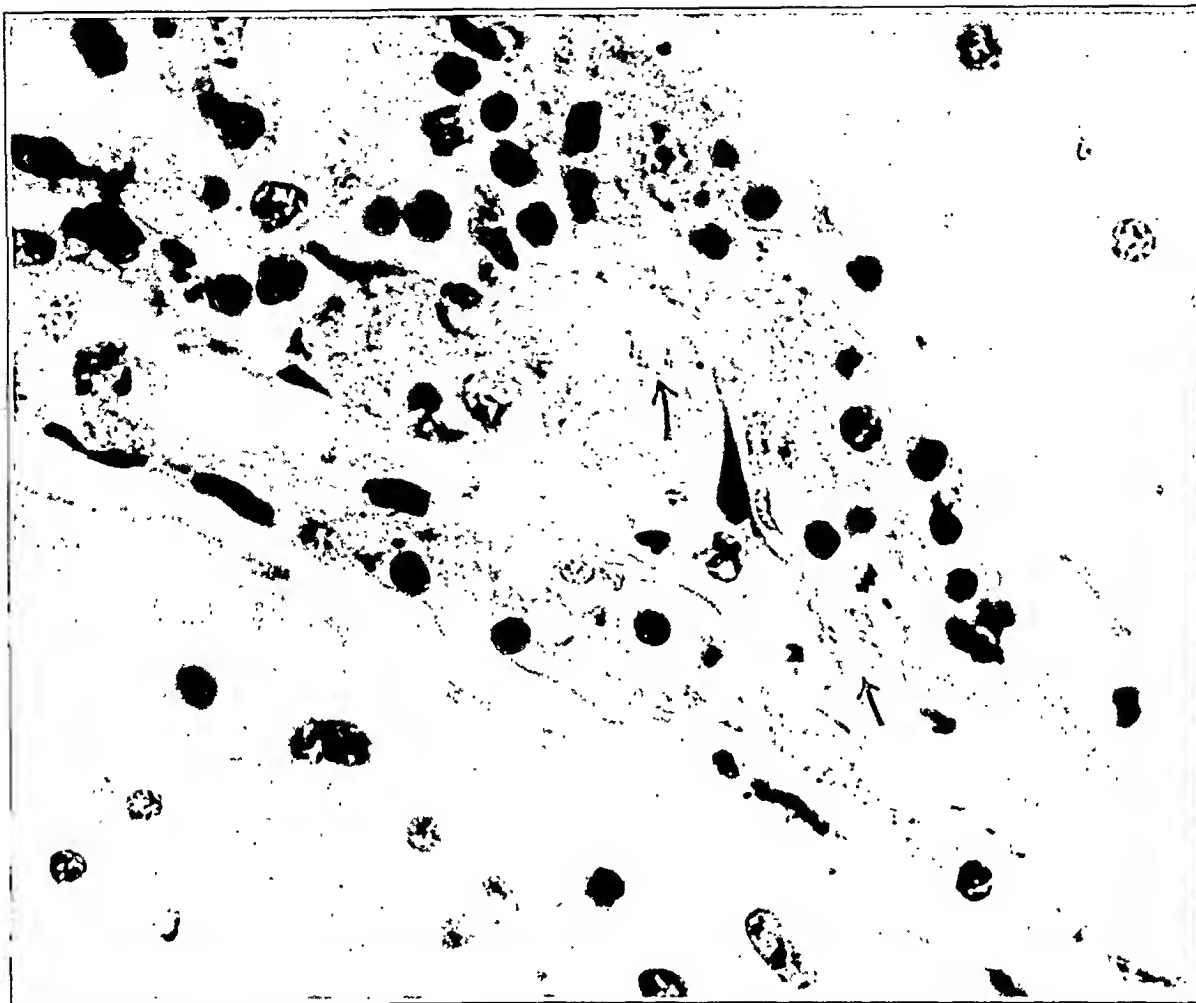


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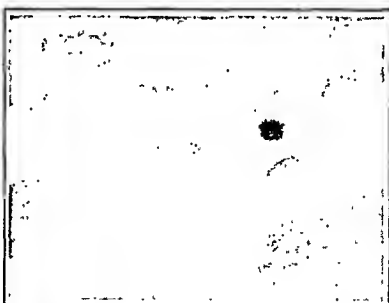




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four above the seventh rib in the posterior axillary line, four over the same rib in the anterior axillary line, three over the vertebral column 1.5 cm. above a line even with the iliac crests, and one 1.5 cm. above the latter. There are linear abrasions over both buttocks. The cervical, axillary and inguinal lymph nodes are enlarged and firm to palpation. A cistern puncture is made and clear fluid under normal (living) pressure removed.

*Primary Incision:* The body is opened by the usual Y-shaped incision extending from both pectoral folds to the xyphoid process and thence to the symphysis pubis. There is a small amount of subcutaneous fat. The pectoral and abdominal muscles are brownish red in color. There is no diastasis of the recti muscles. A small, easily reducible umbilical hernia is present. The umbilical vein and hypogastric arteries are not patent, and repeated sections show no evidence of inflammation.

*Peritoneal Cavity:* There are about 100 cc. of clear straw-colored fluid. No adhesions are present. The liver edge extends 6.5 cm. below the right costal margin in the midclavicular line. The spleen extends 3.5 cm. below the left costal margin in the midaxillary line. The intestinal loops are distended. There is no noticeable abnormality of the mesenteric attachments. The mesenteric lymph glands are firm, injected, and slightly enlarged, the average measuring 0.5 cm. in diameter. The appendix is retrocecal in position and is coiled upon itself, measuring 5 cm. in length. The dome of the diaphragm extends to the fifth rib on the right and the sixth rib on the left. There are no adhesions between the liver and diaphragm. The kidneys can be palpated through the peritoneum and appear enlarged and displaced downward. The bladder is empty and contracted. The uterus, ovaries, Fallopian tubes, broad and round ligaments do not appear unusual.

*Pleural Cavities:* In removing the sternum a well encapsulated circumscribed empyema cavity on the left is accidentally entered. The medial margin of this cavity is 2 cm. to the left of the midsternal line adjoining the outer surface of the pericardial sac for a distance of 4 cm.; its inferior boundary is the diaphragm for a depth of 2 cm.; its superior margin is a strong fibrous wall attached along the chest wall in the midaxillary line as far up as the second rib, and its lateral wall is the inner chest wall. Smears of the creamy yellow seropurulent contents show Gram-positive cocci in long chains. This cavity shows no evidence of communication with the external surface, nor with another larger cavity which contains similar contents and is situated to the left and immediately beneath it. This cavity has many finger-like connecting vestibulae and measures roughly 5 cm. in diameter. The consolidated left lung is pressed closely to the mediastinum. There is evidence of connection between this second cavity and the external surface of the body through the needle punctures mentioned previously. The lower lobe of the left lung above this latter cavity is firmly bound to the chest wall by multiple fine, fibrous adhesions. The pleural surfaces on the left are rough and covered with a shaggy yellowish fibrinopurulent exudate. Those on the right present a marked contrast. They are clear, smooth and glistening and show no evidence of adhesions.

*Mediastinum:* The thymus weighs 9 gm. There is no apparent displacement of the mediastinum. The lymph nodes are firm, slightly enlarged and injected. The right innominate, left carotid and left subclavian vessels are given off in their usual order.

*Pericardial Cavity:* Contains a small amount of clear straw-colored fluid. Smears show no organisms.

# CONGENITAL RHABDOMYOMA OF THE HEART \*

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Congenital rhabdomyomas of the heart are still considered rare tumors. Only two have been reported in the American literature, the last in 1907. Hence the finding of such a tumor, associated in addition with cerebral tuberous sclerosis, is deemed sufficient ground for this report. The increasing number of cases in the foreign literature in recent years affords also an opportunity for a review and consideration of the data now available. A search of the literature has been made, a few early cases, neglected by later writers, have been found and a few more have appeared as case reports since the last review.

## CASE REPORT

*Clinical History:* E. B., a 6 month's old female infant, was admitted with a complaint of "pus in the left side of the chest." Her past and family history were irrelevant. The patient had always been apparently normal and well. The present illness began one month before admission when she began to vomit and had a high temperature. Physical examination showed evidence of pneumonia and empyema. The heart was enlarged and a harsh blowing systolic murmur was heard over the precordium. The neck was stiff and a bilateral positive Kernig sign was elicited. Death occurred nine days after admission and clinical diagnoses of pneumonia, empyema, meningitis, congenital heart and congenital anomaly of the kidney were made. A postmortem examination (A-30-28) was performed one hour after death.

## POSTMORTEM EXAMINATION

*Body:* The body is that of a moderately well developed, poorly nourished, white, female infant, weighing 14 pounds and measuring 70 cm. in length. The circumference of the head is 42 cm. The anterior fontanelle is closed and the sutures are united. Rigor mortis is not present. There is moderate postmortem lividity over the dependent portions. The skin is dry, inelastic and hangs loosely over a small amount of subcutaneous fat. There are multiple, minute, purple, petechial hemorrhagic areas scattered over the abdomen. The hair is light in color, fine in texture and present in a moderate amount over the head. The ears and nasal passages are negative to external examination. The conjunctivae and sclerae are clear. The pupils are equal and regular, measuring 3 mm. in diameter. The hair is shaved from the right temporal region and several needle puncture marks are noted there as well as over the left jugular region,

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lary muscles supporting the anterior cusp of the tricuspid valve. This fits into the space left by the two larger growths previously described, thus causing partial obstruction to the blood flow from the right ventricle to the pulmonary artery. The left auricle is uninvolved. A few smaller areas, approximately 1 mm. in diameter, may be seen scattered throughout the myocardium of the left ventricle, and one small mass measuring 1 mm. in diameter can be seen just below the junction of the two lateral aortic cusps. On cutting into the circular raised area on the interventricular septum from the epicardial surface a yellowish, moderately firm, smooth, slightly bulging surface is revealed. On further section this proves to be only a small cap of a sharply defined tumor mass measuring 3 cm. in diameter. This is grayish yellow in color and firm in consistency. There is a suggestion of a concentric arrangement near the line of gradual merging with normal-appearing myocardium. The myocardium itself is brownish red in color and shows no other abnormality. The thickness of the left ventricle is 7 mm., that of the right 5 mm. The auricles measure 2 mm. in thickness. The valves measure, roughly: tricuspid valve 5 cm., pulmonary valve 2 cm., mitral valve 3.5 cm. and aortic valve 3 cm.

*Lungs:* Right lung weighs 80 gm., the left 110 gm., (normal weight 42 and 38 gm. respectively). The right lung presents a normal yellowish pink appearance and crepitant lappets partially cover the pericardium. The posterior portion of this lung, however, is deep purple in color and firm in consistency, with a mottled appearance given to it by intervening lighter portions. The cut surface here reveals a deep red surface, dry and granular in appearance with thickened bronchioles standing out prominently. The anterior portion exudes a frothy pinkish exudate on pressure.

The left lung is firm, dark red in color and covered with a shaggy yellowish fibrinopurulent exudate. A section near the apex reveals two small circumscribed abscess cavities measuring 1 cm. in diameter. Other sections show marked thickening of the bronchioles, causing them to stand out as white spots against a deep red, meaty, airless background.

*Spleen:* Weight 50 gm., (normal weight 17 gm.). Deep purple in color and slightly less firm than normal. The capsule shows no evidence of thickening or inflammation. The cut surface reveals a deep purple, soft background, against which multiple prominent splenic corpuscles form an evenly distributed speckled contrast. Thin sections are friable and do not retain their shape.

*Gastro-Intestinal Tract:* Opened from the esophagus to anus. The mucosa of the esophagus and stomach does not appear unusual. There is a small amount of bile-stained material in the duodenum. The jejunum, ileum, descending and transverse colons present rare, minute, purple hemorrhagic areas. There is no evidence of gross blood. Peyer's patches do not appear unusual. There are no gross anomalies.

*Heart:* Weighs 90 gm., (normal weight 31 gm.). It measures 6 cm. in its transverse, and 5 cm. in its longitudinal diameter. The epicardium is smooth and glistening and there is no evidence of hemorrhage. There is a bulging mass over the interventricular septum near the auricular ventricular extremity and on closer examination a small, raised circular circumscribed area measuring 1 by 0.2 cm. is located 1.5 cm. from the right lateral margin of the pulmonary artery orifice. The anterior margin of the artery is on a line with the posterior margin of the protuberance. The epicardium does not appear to be interrupted. On opening the right auricle the valve orifice presents a bulbous, yellowish white, firm encapsulated mass 0.8 cm. by 0.9 cm. which projects 2 mm. above the valve ring into the right auricle. A probe can be passed between the cusps and the mass on all aspects except the angle between the anterior and left posterior lateral cusp where the mass is firmly attached and shows evidence of early necrosis, manifested by a dark brown color and softening. There are two small, slightly raised yellowish white areas on the anterior cusp in the midportion, each measuring 2 mm. in diameter. One other area of similar nature is located above the valvular attachment, attached to the auricular wall. On opening the right ventricle, similar masses can be found on the left lateral cusp in close apposition to the larger bulbous mass which is now seen firmly attached to the ventricular wall immediately below the angle of the anterior and left lateral cusps. Its total height is 13 cm., the diameter 0.8 to 0.9 cm. Multiple dome-like, firm, yellowish pink, glistening, smooth projections standing about 2 mm. each above an otherwise grayish white endocardium along the posterior surface of the ventricle up to the pulmonary valve are seen. The largest one on this surface measures 7 mm. On palpation this seems merely to be the surface manifestation of a larger firm mass beneath, which is continuous with the base of the first described bulbous mass. The smaller ones averaging 2 mm. in diameter and projecting about 2 mm. above the surface are grouped near the pulmonary valve orifice where they form a cauliflower-like mass firmly binding the left posterior and right posterior valve leaflets to the heart wall. Several similar small excrescences may be seen between the papillary muscles near the extreme apex of the right ventricle. A large conglomerate mass similar to those described above and measuring 1.5 cm. in diameter is located on the anterior ventricular wall just above the larger papil-

after removal a marble-like pallor is the only unusual feature noted by inspection. The convolutions have the normal appearance; the sulci are of the usual depth. There is no congestion or thrombosis of the vessels and no areas of hemorrhage. The meninges are slightly less translucent than normal, but show no other evidence of inflammatory reaction. The cerebrospinal fluid is normal in character and amount. On palpation there is no undue fluctuation. As the hand is passed lightly over the brain a remarkable unevenness of resistance is discovered. Areas of normal consistence are adjacent to others of stone-like firmness. The distribution of these areas of sclerosis follows no definite pattern except that they are more or less limited by the sulci, rather than spreading in a diffuse manner over portions of several convolutions. Very slight variation in the degree of firmness exists. A few areas of sclerosis may be felt beneath a surface covering of normal cortex. The sclerosis is not more marked on any one part of the brain. The cerebellum and midbrain are not involved and are of apparently normal consistence and structure. After formalin fixation nothing further is found to add to the previous description of the external surface. Two longitudinal sections are made 0.5 cm. to each side and parallel with the median longitudinal fissure. Gray and white matter are well differentiated except in certain areas, which on palpation are extremely firm and found to be continuations of the external areas of sclerosis. These firm cortical areas have either the uniform appearance of white matter or a slightly grayish tinge shading off into white with no definite demarcation. In the white matter of the frontal lobe there is a cluster of firm areas 2 to 3 mm. in diameter. Similar firm areas 1.1 to 0.4 cm. in diameter project from the corpus striatum into the lateral ventricles. No areas of hemorrhage or softening are present. The internal structure of the brain is normal and the ventricular system of the usual size. There is no evidence of ventriculitis or choroiditis. The cerebellum shows no evidence of sclerosis.

*Sinuses:* The venous sinuses are explored. No ante mortem thrombi are found. The endothelial surfaces are smooth and glistening. The dura is stripped from the base of the brain. The bony structure of the base of the skull is normal.

*Middle Ears:* Opened in the routine manner. A thin, yellow purulent fluid is found in both middle ears, which on smear shows Gram-positive cocci in chains. The tympanic membranes are slightly opaque but intact. A slight amount of the same fluid is found in both mastoids but there is no evidence of bone necrosis.

*Pancreas:* Normal in shape, size and consistency.

*Liver:* Weight 350 gm., (normal weight 200 gm.). It is mottled yellow and purplish red in color. The capsule is not thickened. Cut section shows a fine streaking of congested vessels forming a network for radiating yellowish white linear areas against a yellowish red background. Uniformly distributed, minute grayish spots add focal necrosis to the possibilities of fatty infiltration and passive congestion.

*Gall-Bladder:* Moderately distended with clear yellow bile which is easily expressed through the ampulla of Vater without difficulty. The mucosal surface is smooth and velvety. The duct system presents no abnormalities.

*Kidneys:* Right weighs 60 gm., left 70 gm., (normal weight 26 and 25 gm. respectively). They are mottled, yellowish red to grayish red in color, with darker areas which bulge slightly above the surface, distributed irregularly. These areas are fluctuant and vary in size from 1 mm. to 7 mm. The capsules strip with ease. The parenchyma everts on cutting. The cortex and medulla are well defined, but irregular. The medullary pyramids in some areas are displaced by firm, yellow, granular-appearing material. Several cystic areas are encountered which contain clear straw-colored fluid. The pelves are smooth, white and glistening. There is no evidence of uric acid deposits. The ureters are of average caliber and pursue their usual course to the bladder.

*Adrenals:* Normal in size, shape and location.

*Genitalia:* The external genitalia are normal. The uterus, Fallopian tubes and ovaries are in their normal location.

*Aorta:* Opened throughout its length it reveals no evidence of atheromatosis or degeneration.

*Organs of Neck:* The thyroid appears normal in size, shape and consistency.

*Bone Marrow:* The osteochondral junctions are even and regular. The line of provisional ossification is not widened. The bone marrow itself is deep red in color.

*Spinal Cord:* Removed from the cervical to lumbar region. There is no evidence of extra- or subdural hemorrhage.

*Brain:* Weight 780 gm., (normal weight 659 gm.). The usual mastoid to mastoid incision is made and the scalp flaps reflected. The superficial fascia and galea aponeurotica present no unusual features. The bony structure of the head is normal. There are no exostoses and no evidence of cranio tabes or osteoporosis is present. The calvarium is removed by sawing through the skull in the occipitofrontal circumference and separating it from the dura. The internal structure of the skull is normal. There are no hemorrhages involving the dura which is cut in the same line with the skull and reflected medially. The falx and tentorium are intact with no evidence of old or recent hemorrhage. The vein of Galen is not thrombosed and presents its normal appearance. The brain is removed by cutting the nerves and other attachments in order. On examination

places failing to merge with the wall. On closer examination it is seen that these processes are transversely striated by rows of delicate granules. With suitable stains these granules stand out clearly, and the processes as well as the walls of the spaces show numerous definite cross-striations. The location of the spaces within the cells, as described by previous observers, is noted.

The cells generally have but one nucleus, occasionally two, and rarely three and four nuclei are found in a single cell. Sometimes clear spaces surround the nucleus and in such cases the processes are generally short and plump. A single nucleolus is usually present and occasional evidence of direct division is noted. No mitotic figures are seen.

The sections were compared with a specimen of the tumor described by Wolbach<sup>1</sup> and found to resemble it so closely in both the size of the elements and the finer histological details, that the following description is taken from Wolbach's paper and given freely as follows:

*"The tumor is composed largely of cells with striated fibrils, in some instances with the full detail of normal fibrils, in most instances in the form of granules united by delicate fibrillary material. The granules stain deeply with iron hematoxylin. With phosphotungstic acid hematoxylin the granules stain a deep blue, the fibrils pale brownish or gray. With Mallory's connective tissue stain the granules stain red, the fibrillary substance blue.*

Cells showing least structural detail contain clusters of paired granules having the staining properties of centrioles, and like centrioles are embedded or connected by centropasm or centrolin. Individual granules (centrioles) are in the neighborhood of 0.2 microns in diameter. Larger bodies are probably clusters of unresolvable granules. The granule clusters rarely are as large as 2 microns in diameter; 1 to 1.5 microns diameter are frequent, but the majority are 0.5 to 1 micron.

Next in order of complexity are cells with great masses of paired granules. Other cells show a few fine fibrils connecting the granules and the beginning of an orderly arrangement in process toward the formation of the segmented fibril. The most completely matured cells are filled with segmented fibrils in bundles running in all directions. A few contain fibrils with alternate broad and narrow cross-striations but most of the fibrils have the appearance of granules strung on fibrils uniformly apart at distances of 1.6 microns and arranged in phalanx formation. However, scattered among the segmented fibrils are clusters of centrioles composed of from two to many members, often in diploid form. These centriole clusters have all the characteristics of those in the least differentiated cells and may be interpreted as clusters, the members of which have failed to disperse.

The tumor cells vary from 50 to 200 microns in diameter. The majority of the nuclei lie between the limits of 9 by 7 microns and 13 by 10 microns."

*Anatomical Diagnoses:* Congenital rhabdomyoma of the heart, cerebral tuberous sclerosis, congenital polycystic kidneys, meningitis, bronchopneumonia, lung abscess and empyema due to streptococcus hemolyticus.

### MICROSCOPIC EXAMINATION

*Heart:* After the heart was partially sectioned, vertical slices, approximately 2 mm. in thickness, were taken through the large tumor in the interventricular line, from a smaller tumor nodule in the right ventricle, and from an area in the right myocardium, apparently free from tumor. These were fixed in Zenker's fluid. A thin section from the largest nodule was fixed in absolute alcohol and a similar section was teased on a slide and examined immediately. The Zenker-fixed material was stained by the following methods: hematoxylin and eosin, phosphotungstic acid hematoxylin, Mallory's connective tissue stain (phosphomolybdic acid, anilin blue, orange G after acid fuchsin), and Van Gieson's method. The section fixed in absolute alcohol was stained by Best's carmine stain for glycogen.

The two sections taken from the tumor nodules show little variation. There is no trace of normal tissue. Under low power numerous large vacuolated spaces varying from round to oval in shape and irregular in size dominate the picture, giving to the sections a loose, sponge-like appearance. Numerous heavy connective tissue trabeculae, in which lie blood vessels, course through the tumor, giving off finer and more delicate branches which ramify among the large vacuolated spaces. Thick protoplasmic walls surround the spaces and between them in places a thin, delicate connective tissue reticulum can be found. This can best be seen with the connective tissue stain. There is, however, no relation between this connective tissue reticulum and the walls of the vacuolated spaces.

Some of the spaces are empty. Others show cells with many processes lying within the clear spaces. The cells lie usually in the center of the spaces, or, less often, against the wall. There is great variation in the size and shape of the cells, depending in part on the size of the space within the cell. The processes run from the center protoplasmic mass in bizarre and irregular fashion. Sometimes they anastomose richly and divide into finer elements until they merge with the wall. Often the processes are short, plump and few in number, in

nuclei. These cells are somewhat more numerous in the deeper layers of the cortex among the large pyramidal cells and polymorphous nerve cells. The third type of reaction indicates a secondary calcification with many deep blue calcified areas, some of which are slightly laminated. In the areas of sclerosis a few nerve cells are seen, but they are smaller than normal, have a distinctly compressed appearance and stain a deep blue. Edema is a prominent feature in all the sections but appears to be more marked in some of the areas of sclerosis than in others. The nerve cells and many of the large glia cells are surrounded by wide clear spaces which are traversed by nerve fibers or delicate glia fibrils. The perivascular spaces are also considerably wider than normal and many of the capillaries are narrow and appear compressed. The sections from portions with no gross pathology are normal except for moderate edema and chronic meningitis already described. In one section there is slight evidence of choroiditis, but no ventriculitis.

#### REVIEW OF LITERATURE AND DISCUSSION

Only thirty-three undoubted cases (exclusive of our own) of the multiple type and eight of the single type of rhabdomyoma of the heart have been reported. In addition, one case of replacement of the entire myocardium by a diffuse rhabdomyoma<sup>2</sup> has recently been published, thus making a total of forty-one cases already on record.

The earlier cases of Kantzow and Virchow,<sup>3</sup> Skrzeczka,<sup>4</sup> Rieder,<sup>5</sup> and Justi,<sup>6</sup> rejected in the papers of Seiffert<sup>7</sup> and Wolbach,<sup>1</sup> were again reviewed and likewise rejected. The case of Kantzow and Virchow proved to be hyperplasia in the region of a gummatous process in the heart. Skrzeczka's case showed so much postmortem change that recognition was impossible. Rieder's case was regarded by Weigert as a true congenital malformation and not as a tumor. The description given by Justi was too poor to permit judgment.

Kennard<sup>8</sup> recently reported the finding, in a man 41 years of age, of a primary tumor of the heart associated with porencephalus. The heart weighed 750 gm. The tumor was situated in the apex and involved the wall of the left ventricle. Kennard's histological description does not include the few characteristic details which have been repeated with almost monotonous regularity by every author.

The third Zenker-fixed section, taken from the right myocardium, showed essentially normal myocardium, except for two widely separated islands of tumor cells. These consist of from four to eight vacuolated spaces, surrounded by zones of dense connective tissue. The spaces are empty and the walls show definite cross-striations. These small isolated areas in the myocardium are similar to those described by Rehder.<sup>25</sup> The sections stained by Best's method show large amounts of glycogen in the form of fine droplets filling the spaces and gathered thickly along the cell processes.

*Spinal Cord:* The nerve cells stain fairly well; however, several of the nuclei are eccentrically located. Nissl bodies stain darker than the nuclei and are located near the periphery in all cases. The glial cells are present in about their usual proportion. There is no evidence of gliosis, hemorrhage or areas of softening. The meninges are not thickened and there is no evidence of exudate.

*Brain:* There are fifty sections representing every part of a complete sagittal section 0.5 cm. to one side of the longitudinal fissure. The sections may be divided into those showing sclerosis grossly and those from apparently normal areas. There is a chronic and acute meningitis evident in both sets of sections. The meninges are slightly thickened by fibrous tissue and organizing exudate. Polymorphonuclear leucocytes occur in rather large clusters in more or less isolated areas of the meninges, chiefly but not exclusively around the meningeal vessels. Plasma cells and macrophages are fairly numerous. The reaction extends to a slight extent along the pia and is found in the perivascular spaces of the small vessels in the nervous tissue. A few cocci in pairs and short chains are occasionally seen among the polymorphonuclear leucocytes. Several of the capillaries are entirely filled with cocci, indicating postmortem growth.

The sections from the sclerosed areas show three distinct types of reaction. First, there is a marked gliosis forming a dense network of fibrils with fewer nuclei than are seen in normal areas. The increase in the fibers of the tangential network is more prominent than that in any other areas, with the exception of the sclerosed projections of the corpus striatum into the ventricles, which are covered with a similar dense network. There are different degrees of gliosis shading off almost imperceptibly to normal. Second, there are areas of less marked gliosis but with many immature glia cells of very large size with many fine spider-like processes and one to three small oval



The apparent rarity of this tumor demands some explanation. A number may be missed in the absence of routine microscopic examinations. Steinbiss offers a somewhat ingenious explanation. Since so many of these cases are associated with tuberous sclerosis, and since it is now known rhabdomyomas of the heart are not confined to the first few years of life, he argues that the highest incidence would probably occur in institutions for the care of the feeble-minded. His own experience seems to bear that out, for in ten years in such an institution Steinbiss found twenty-one cases of tuberous sclerosis, six of which were associated with rhabdomyoma of the heart. If, in such institutions, more autopsies were done and if of these fewer were limited to the brain, more cases of rhabdomyoma of the heart might be found.

Most of the earlier reported tumors occurred in individuals under 3 years of age, and that observation was frequently stressed as proof of their congenital nature. A survey of the accompanying tables shows that twelve of the forty-one undoubted tumors were found in patients over 3 years of age, the oldest being 35 years. The sex is predominately male, but the series is naturally too small to permit any conclusion. Poor nutritional states were found in many instances, and several early authors thought of some relation between the poor nutritional state and the heart and brain pathology. A few patients, however, were in an excellent state of nutrition. Several showed marked physical retardation (Jonas,<sup>17</sup> Steinbiss<sup>18</sup>). The tumor described by Jonas was in a 6 month's old male infant who weighed 3.5 Kg., and measured 55 cm. in length, approximately a little more than the measurements and weight of a normal full-term infant. The third case of Steinbiss was a 10 year old male who weighed 10 Kg., and had no teeth.

The nature and location of the large spaces in the microscopic picture were for a time a subject of debate. Von Recklinghausen,<sup>18</sup> who described the first case, arrived at no conclusion, but thought that they might be lymph spaces (similar to the macroglossia of Virchow), blood spaces or muscle tubes of pathological origin. Virchow<sup>19</sup> stated "whether we shall regard the cavernous spaces as lymphatic or just as serous cavities, cannot be settled. The main substance of the tumor was muscular, however, and apparently a result of hyperplasia of the myocardium." Hlava<sup>20</sup> regarded the spaces as artefacts due to the effect of alcohol fixation and placed their position as in-

Hence, his case will not be included in this series. Bradley and Maxwell<sup>9</sup> recently placed on record a tumor which they believe is the first of its kind to be reported. The patient was a man of 62 years who had complained of pain in the upper left chest for five to six weeks before death. A tumor mass was found in the pericardial sac, in the heart wall and in the mediastinum. The heart and mass weighed 1900 gm. The brain was not examined. They believed that they were dealing with a rhabdomyosarcoma, primary in the heart. Their microscopic description does not correspond to the other accepted cases, and the metastases alone serve to set this case apart from those discussed here.

Three cases, about which no details could be obtained but which appear to be reliable, are included in a separate section of the accompanying table. Askanazy<sup>10</sup> in a discussion following Seiffert's report of his second case,<sup>11</sup> stated that he had investigated a case in which he had found glycogen. This case he apparently has not published. Goldstein<sup>12</sup> mentioned a case seen by Lucke at the Blockley Hospital. No details accompany the statement. Steinbiss<sup>13</sup> described the microscopic specimen of an incorrectly diagnosed heart tumor which came to his attention. This he had no hesitancy in calling a typical rhabdomyoma. Since Steinbiss recorded six other cases and described with great accuracy the histological details, this accidental finding should be listed as an additional unclassified case.

One case in animals<sup>14</sup> has been reported. Multiple rhabdomyomas were found in the heart of a well developed 6 month's old female pig. The brain and kidneys were not microscopically examined, but they were grossly negative.

Wolbach mentions in his paper, but does not include in his table, a case reported by Billard<sup>15</sup> which occurred before the days of histological examination. There were three small tumors in the anterior wall of the left ventricle and in the interventricular septum of an infant, 3 days of age. Billard's case was mentioned by Kolisko<sup>16</sup> but omitted by Seiffert. Wolbach thought that Billard's case was probably one of multiple rhabdomyomas.

Seiffert's second case was reported a few months after his first, and was neglected by all later writers. The inclusion of this case in the table, as well as complete tabulation of all authentic cases, will account for the discrepancy in the total number of cases as compared with previous reviews.

TABLE I

*Multiple Forms in Cases of Rhabdomyoma of the Heart*

Author	Heart	Brain	Other Organs	Age	Year	Remarks
1. von Recklinghausen <sup>18</sup>	Nodules in both ventricular walls	Tuberous sclerosis	Negative	Newborn	1863	
2. Virchow <sup>19</sup>	Numerous nodules in both ventricular walls	Not mentioned	Liver large. Multiple tumors in skin	Newborn	1864	Well nourished. Omphalitis
3. Kolisko <sup>10</sup>	Small nodules in right conus arteriosus and in anterior wall of right ventricle and septum	Negative		2 mos.	1887	Found dead
4. Cesaris-Denel <sup>21</sup>	Large tumor in apex of right ventricle. Many small nodules in left ventricle wall and septum	Tuberous sclerosis	Small nodules of embryonic renal tissue without glomeruli	3 yrs.	1895	
5. Seiffert <sup>7</sup> (Case 1)	Large tumor at apex, small nodules in myocardium and septum	Negative	Kidney cysts	20 mos. (male)	1900	Rickets
6. Seiffert <sup>11</sup> (Case 2)	Multiple small tumors in myocardium	Not mentioned	Not mentioned	7 mos.	1900	Rickets
7. Rothe <sup>43</sup>	Multiple tumors. No details	Tuberous sclerosis	Multiple tumors of breast	?	1901	
8. Ponfick <sup>22</sup> (Case 1)	Large nodules in right ventricle, nodules in both ventricular walls	Tuberous sclerosis	Not mentioned	7 mos. (male)	1901	Bronchopneumonia. No psychical disturbances
9. Ponfick (Case 2)	Large nodule in anterior wall of right ventricle, numerous nodules in left	Tuberous sclerosis	Not mentioned	3 yrs. (female)	1901	Chronic furunculosis. Emaciation

tracellular. Kolisko<sup>16</sup> believed the spaces lay between cells and so located them in a plate accompanying his paper. Cesaris-Demel<sup>21</sup> thought the spaces were intercellular, similar to spaces he found between anastomosing cells in the hearts of human embryos. Seiffert<sup>7</sup> was the first to produce embryological evidence to locate the spaces. Ponfick<sup>22</sup> and Knox and Schorer<sup>23</sup> agreed that the spaces were intracellular in position but offered no additional proof. The evidence of Seiffert was convincing, but it remained for Wolbach, with the aid of the phosphotungstic acid hematoxylin stain, to offer conclusive evidence of the intracellular position of the spaces.

Cesaris-Demel was the first to call attention to the many-processed cells lying within the spaces and these he called spider-like cells. Seiffert likened the cells to spiders in their webs, and the characteristic cell of this tumor has since been called the "spider cell." Steinbiss objects to this designation and suggests that a more related comparison would be more suitable. He compares the tumor cells to cells of *Tradescantia*. Running from the primordial tube which lies beneath the cell membrane are plasma threads which anastomose richly with one another until they unite in a larger plasma mass near to the center, leading to the nucleus. Between the threads are vacuoles filled with fluid. This he uses to further the comparison by mentioning the glycogen-filled vacuoles in the tumor cells.

The contents of the vacuolated spaces were not definitely recognized by the earlier writers. Although some looked upon them as evidences of degeneration, Marchand,<sup>24</sup> in a discussion to Seiffert's second paper (1900), reasoning by analogy to fetal muscle, suggested that glycogen might be present. In the same discussion Askanazy stated that he had demonstrated glycogen in small droplets in a case which he had occasion to study. Although Seiffert had been unable to demonstrate glycogen clearly, he was convinced that it was present in the spaces. In 1914, both Rehder<sup>25</sup> and Mönckeberg<sup>26</sup> were able to demonstrate glycogen in the tumor cells. Later writers also reported a similar finding, and I was able to find glycogen in large amounts in my case. Bundschuh<sup>27</sup> found small amounts of fat in fine droplets in the muscle cells at the periphery of the tumor. Kawamura<sup>28</sup> described fat in small and large droplets in the vacuoles and fibrils and Mittasch<sup>29</sup> mentioned that his cases showed both fat and glycogen. Steinbiss stated definitely that the vacuoles were free from fat. Walbaum<sup>30</sup> could find no fat in the myocardium of fetuses and young infants.

TABLE I (cont.)

Author	Heart	Brain	Other Organs	Age	Year	Remarks
20. Mönckeberg <sup>20</sup>	Multiple nodules, especially at points of attachment of mitral and tricuspid valves	Firm consistency of cortex. (No microscopic examination)	Absence of right kidney, ureter and vessels	14 mos.	1914	Symptoms of "brain affection" before death. Recovering from measles
21. Rehder <sup>21</sup>	Large nodule at apex, reaching to septum. Small ones in right ventricular wall. Pulmonary artery obstructed	Grossly negative. (No microscopic examination)	Grossly negative	Newborn	1914	Well developed and nourished
22. Ribbert <sup>22</sup>	12 nodules in myocardium	Tuberous sclerosis	Multiple kidney cysts	1 yr.	1915	Died of diphtheria
23. Mittasch <sup>23</sup> (Case 1)	Nodule in right auricle. Numerous nodules in lower third of median wall of conus arteriosus and in lower half of auricular septum	Tuberous sclerosis	Kidney cysts and nodules of undifferentiated renal tissue	4 mos.	1922	Measles, pneumonia
24. Mittasch (Case 2)	Multiple nodules. No details	Tuberous sclerosis	Kidney tumors	14 yrs. (male)	1922	Acute yellow atrophy
25. Mittasch (Case 3)	Multiple nodules. No details	Tuberous sclerosis	Kidney tumors. Angiomyolipoma of liver	31 yrs. (male)	1922	Pneumonia and sepsis
26. Steinbiss <sup>13</sup>	Nodules in left ventricular wall, septum and papillary muscle of right ventricle	Tuberous sclerosis	Fibro-epithelioma of skin over face	5 yrs. (male)	1923	Idiot. History of epileptiform seizures

10. Bonome-Cagnetto <sup>42</sup>	Multiple tumors. No details	Tuberous sclerosis	Not mentioned	18 mos.	1895	Rickets
11. Knox and Schorer <sup>23</sup>	Large pedunculated tumor in left ventricle. Numerous small ones	Not mentioned	Negative	7 mos.	1906	Malnutrition
12. Abricossoff <sup>32</sup>	Multiple nodules in both ventricular walls. Two large nodules in anterior wall of left ventricle	Tuberous sclerosis	Negative	3½ yrs.	1909	Died fourth day of scarlet fever
13. Kaufmann <sup>44</sup> (Case 1)	Multiple nodules	Tuberous sclerosis		3 yrs.	1922	
14. Kaufmann (Case 2)	Multiple nodules	Tuberous sclerosis	Multiple kidney tumors	7 yrs.	1922	
15. Schulgin <sup>31</sup> (Case 1)	Multiple tumors in both ventricular walls	Tuberous sclerosis	Kidney tumors	6 days	1913	
16. Schulgin (Case 2)	Multiple tumors in both ventricular walls	Tuberous sclerosis		6 yrs.	1913	
17. Bundschuh <sup>27</sup>	Nodules in left ventricle, apex and septum. One nodule composed mainly of fat	Tuberous sclerosis	Kidney tumors and cysts. Adenoma sebaceum	2 yrs. (female)	1912	Well nourished. Convulsions
18. Kawamura <sup>28</sup>	Multiple nodules in both ventricular walls and especially in septum. One in left auricle	Tuberous sclerosis? Brain not examined	Kidney tumors. Congenital anomalies in pancreas, esophagus and rectum	4 yrs. (female)	1913	Idiot. Epileptiform convulsions
19. Jonas <sup>17</sup>	Multiple nodules in both ventricular walls. Small nodule in right auricle	Tuberous sclerosis	Flare lip. Cleft palate	6 mos. (male)	1912	Poorly nourished, underweight, underdeveloped

TABLE II  
*Solitary Forms in Cases of Rhabdomyoma of the Heart*

Author	Heart	Brain	Other Organs	Age	Year	Remarks
1. Hlava <sup>20</sup>	Tumor in left ventricle	Not mentioned	Negative	14 days	1886	Sudden death
2. Wolbach <sup>1</sup>	Tumor in right ventricle arising from septum and posterior median papillary muscle	No tuberos sclerosis	Spina bifida, multiple neuroglia nests in cortex of brain and spinal meninges	10 mos. (female)	1907	
3. Ehmrooth <sup>45</sup>	Large nodule comprising almost entire left ventricle wall	No gross changes. (No microscopic examination)	Grossly no changes in other organs	7 mos.	1911	Well nourished. Died suddenly after coughing
4. Hisinger-Jägerskiöld <sup>40</sup>	Nodule at apex, size of hen's egg. Comprises greatest portion of the posterior ventricle wall and septum	Not mentioned	Not mentioned	7½ mos.	1916	
5. Amersbach and Handorn <sup>35</sup>	Encapsulated tumor at atrioventricular border, forming a part of the anterior wall of left ventricle, reaching into the septum	No tuberos sclerosis	Negative	7 days (male)	1921	Full term, cyanotic at birth. Died with signs of heart failing slowly
6. Steinbiss <sup>13</sup>	Nodule size of thumb-nail at apex of left ventricle. Heart atrophic	Tuberos sclerosis. Solitary nodules in temporal lobes. Calcified nodules in ependyma	Numerous solitary cysts. Tumors in renal cortex	35 yrs.	1923	Cachectic epileptic
7. Omodei-Zorini <sup>47</sup>	2 cm. tumor in right ventricle wall near apex	No details	No details	2½ yrs.	1923	
8. Sikl <sup>48</sup>	No details available	No details	Hare lip. Cleft palate	9 wks. (male)	1925	Clinical diagnosis of valvular disease

27. Steinbiss (Case 2)	Numerous nodules, especially in right ventricular wall. Larger nodules at left apex	Tuberous sclerosis	Kidney tumors	8 yrs. (male)	1923	Epilepsy. Death in seizure
28. Steinbiss (Case 3)	Multiple nodules in both ventricular walls	Tuberous sclerosis	Kidney tumors. Adenoma sebaceum	10 yrs. (male)	1923	Idiot. Death in marasmus. Weight 10 Kg. No teeth
29. Steinbiss (Case 4)	Nodule in septum and cicatrix-like nodule in left myocardium	Tuberous sclerosis	Kidney tumors and cysts	16 yrs. (male)	1923	Idiot. Death in seizure
30. Steinbiss (Case 5)	Two pea-sized nodules in right auricle	Tuberous sclerosis	Kidney tumors	21 yrs. (female)	1923	Mental deterioration since puberty
31. Uehlinger <sup>33</sup>	Numerous nodes in left myocardium septum. Large node at foot of anterior papillary muscle	Leptomenigitis. Chronic fibrosclerosis. (No microscopic examination)	Negative	20 yrs. (male)	1925	Tetanus
32. Riedmatten <sup>51</sup>	Multiple nodules	Tuberous sclerosis		1½ yrs.	1904	
33. Berger and Vallée <sup>50</sup>	Multiple nodules	Brain not examined	Polycystic kidneys	2 yrs.	1930	Epileptic. Well nourished
34. Farber	Multiple large and small masses chiefly in right ventricle	Tuberous sclerosis	Polycystic kidneys	6 mos. (female)	1930	Died of <i>Strept. hemolyticus</i> bacteremia. No history of heart or brain disturbances



The conclusion that the cells of this tumor probably arise from Purkinje fibers was drawn by many authors (Knox and Schorer,<sup>23</sup> Kawamura,<sup>28</sup> Schulgin,<sup>31</sup> Mönckeberg,<sup>26</sup> Abricossoff,<sup>32</sup> Uehlinger,<sup>33</sup> and Berger and Vallée<sup>50</sup>). Retzer and Aschoff,<sup>34</sup> although not reporting cases, gave support to this view. This theory sounds logical at first glance. The vacuolated appearance, the differentiation into fibrils at the periphery, the restriction of the cross-striations to these fibrils and the richness in glycogen content of the Purkinje cells, all point to similarity to the tumor cells. Tawara found in the ventricular muscle occasional fibrils which are similar to the terminal processes of the conduction system and Rehder found vacuolated Purkinje fibrils in the myocardium of a 46 year old man and a 13 year old boy. These are similar but smaller than the rhabdomyoma cells. Rehder includes an illustration of the 46 year old case in his paper and argues that the location of the tumor in the reported cases, in the anterior ventricular wall, septum, trabeculae and papillary muscles, situated mostly subendocardially, plus the similarity to Purkinje fibrils, permit the conclusion that the tumor arises from the conduction system. Steinbiss, however, points to the fact that the tumors are often found where the conduction system cannot be demonstrated, and further, that if this tumor were a malformation of the conduction system, a disturbance of conduction during life, especially in marked cases, could be expected. But this finding has never been noted. Amersbach and Handorn<sup>35</sup> proved to their satisfaction that there is no connection between the conduction system and the tumor by tracing out both structures in serial section. Rehder believes that the tumor arises from a primitive layer (Mutterboden) common both to the Purkinje fibrils and the myocardial cells before the differentiation into the two muscle systems takes place. On the basis of the available evidence, this view appears to be the most logical explanation.

The true status of the tumor was not settled for a time. Kolisko first stated that the cells are embryonic in type. Cesaris-Demel compared the tumor spaces to spaces he found between anastomosing cells in the hearts of the human embryos. Later, Seiffert, on the basis of the work of Felix<sup>36</sup> on striated muscle in the embryo, studied the embryonic muscle cells of frogs, cats and the human fetus and so compared the tumor cells to embryonic heart muscle cells during the stage of fibril formation, when the fibrils are limited to the periphery

TABLE III  
*Unclassified Forms in Cases of Rhabdomyoma of the Heart*

Author	Heart	Brain	Other Organs	Age	Year	Remarks
1. Schmincke <sup>2</sup>	Diffuse nodules entire myocardium. Right myocardium 1.3 cm. Left myocardium 1 cm.	Negative	Negative	Newborn	1922	
2. Askanazy <sup>10</sup>	No details	No details mentioned in discussion				
3. Lucke <sup>12</sup>	No details	No details mentioned by Goldstein				
4. Steinbiss <sup>13</sup> (Case 7)	No details	No details mentioned			1923	
5. Hieronymi and Kukla <sup>14</sup>	Multiple nodules	Negative	Negative	6 wks. (female pig)	1921	Well developed
6. Billard <sup>15</sup>	Multiple nodules. (No microscopic)	Not mentioned		3 days	1828	
7. Taruffi <sup>22</sup>	No details					

tions have also given a means of diagnosis of tumors of striated muscle when differentiation is low and cross-striations are not present.

The Cohnheim-Ribbert theory of embryonic rests, activated in response to mechanical disturbances, was early introduced as an explanation of the occurrence of the rhabdomyoma. Seiffert stresses the great opportunity for tissue injury, though not gross in character, in an organ with a development so complicated as that of the heart. Steinbiss takes issue with Seiffert's stand, pointing out that the very rarity of the tumor is the greatest evidence against such a hypothesis. Ribbert <sup>38</sup> later states that he does not believe the vacuolated areas he found strewn through the myocardium of his case are rests of embryonal musculature, but that they are independent shoots (sprouts). The areas he describes are always surrounded by connective tissue and have a larger circumference and thicker striations than in the embryo.

Rehder believes the rhabdomyoma is a tissue malformation and not a true tumor. He calls it a hamartoma (Albrecht <sup>39</sup>) and not a hamartoblastoma, and so would not classify it with true muscle tumors such as rhabdomyoma of the esophagus and bladder. Schmincke <sup>2</sup> also regards the rhabdomyoma of the heart as a malformation, *i. e.*, a hamartoma. He describes it as the end of a continuous chain, at the other end of which are the smallest areas of embryonal muscle tissue in the heart of a newborn. Steinbiss also takes issue against the classification of this tumor as a true neoplasm. He points out that the form of the embryonal cell is retained but the size is greatly increased. There is a retardation in the stage of development, but hypertrophy of the individual elements. With this hypertrophy the cells reach a degree of tissue maturity and proportional to this maturity they lose their potentiality for growth beyond a certain point. This differentiates them from the embryonal rests (Ribbert), which have much greater prospective potentiality, and so metastases and rapid growth are unlikely. No true progressive changes have been described. Occasional mitotic figures, as noted by Bundschuh and Abricossoff can be compared to a similar multiplication in normal striated muscle. The later fate of the tumor also argues against a true neoplasm. Steinbiss describes these changes as regressive in character with nuclear changes, the process becoming plump, the cytoplasm granular and the vacuoles smaller.

of the cell, while the central portion is filled with a homogeneous substance which does not stain. The part of the sarkoplasm which surrounds the tumor cell remains undifferentiated. Knox and Schorer laid stress upon the clear spaces in the embryonal cells and in modified muscle cells found in adult hearts. Wolbach mentions that he saw similar cells in the moderator band from an infant's heart. In a number of autopsies Rehder noted as an incidental finding small vacuolated areas in the myocardium, which he compared to Seifert's description of the heart muscle of a 2 month's old human embryo.

Further studies by means of staining reactions established fully the true nature of the tumor. Wolbach, in 1907, using the phosphotungstic acid hematoxylin, and anilin blue stains, was able to demonstrate in the more differentiated portions of his tumor the alternating thick and delicate striations, the sarcous elements and the membranes of Krause in normal muscle, the former staining red with the connective tissue stain, and the latter blue. In the same paper he describes in the large cells with abundant protoplasm, clusters of deeply staining granules without any definite arrangement or connection with one another. These granules are sometimes in pairs, biscuit-shaped, with the flat sides apposed, giving an appearance strongly suggestive of centrosomes. The granules, when stained with the aniline blue stain, are colored a brilliant red with the acid fuchsin. Occasional granules in groups of two to many are connected by delicate fibrils in a more or less orderly fashion. Wolbach concludes that "this is evidently the beginning of muscle fibril formation." This was the first observation of this kind, and many years later (1928) on the basis of further study of this tumor, and a malignant rhabdomyoma of skeletal muscle, Wolbach<sup>37</sup> was able, by reconstructing certain sequences, to show the rôle of the centrosome in myofibril formation. In his later paper Wolbach describes the apparent sequence as follows: "Multiplication of centrioles and centriole clusters; dispersion of centriole clusters, dispersion of centrioles, probably continued division of centrioles and fibrils." Based upon staining reactions and apparent morphological sequences, Wolbach gives the following interpretation: "The granules of centriole origin give rise to the dark disc or 'Q' band; the fibrillary material contracts into the 'Z' band (Krause's membrane)." In addition to clarifying the origin of this tumor, Wolbach's observa-

berous sclerosis will be given here. The subject will be treated at length in a later paper from this laboratory. A large literature <sup>41</sup> has grown up on the subject of cerebral tuberous sclerosis in the past thirty years and many more cases of tuberous sclerosis than rhabdomyoma of the heart have been reported.

Tuberous sclerosis in instances of rhabdomyoma of the heart was first noted but not described by von Recklinghausen. Cesaris-Demel reported the association in his case, but did not describe the condition. Ponfick was the first to call attention to the association of rhabdomyoma of the heart and tuberous sclerosis and described a diffuse fibrillary gliosis limited to the gray matter. He believed the two conditions to be congenital and in some way related. Bonôme-Cagnetto's two cases <sup>42</sup> were also associated with tuberous sclerosis, and he sought an explanation and possible common factor in the evidence of fetal malnutrition with secondary vascular degeneration. Further studies were made by Jonas, Ribbert, and most of the later writers. An examination of the accompanying tables shows that associated with the multiple type of rhabdomyoma, tuberous sclerosis was present in 24 out of 28 cases where the head was mentioned, and with the single type, 1 out of 4 cases.

Also associated with rhabdomyoma of the heart, but to a lesser degree, have been reported kidney tumors <sup>49</sup> of various types and titles, *e. g.*, angiomyosarcoma, angiolipoma and renal adenoma; cysts of the renal cortex, rests of embryonal renal tissue without glomeruli, and adenoma of the skin. The accompanying tables record the association in each instance. Certain other developmental anomalies such as multiple neuroglia rests in the meninges, congenital malformation of the pancreas, <sup>28</sup> as well as the more frequent structural anomalies such as harelip, have also been reported. However, by far the most constant association has been tuberous sclerosis. All six cases reported by Steinbiss had tuberous sclerosis. One of these cases (5) showed degeneration of the cerebral process, with calcification, widespread ossification and formation of marrow spaces.

NOTE: I wish to thank Dr. G. A. Bennett for the photomicrographs and Dr. O. Gates for the pictures of the brain.

Gradually the mass decreases in size and is replaced by connective tissue, to be noted eventually as scars of unknown origin in the myocardium. In one case Rehder reports calcification in a portion of his tumor. He concludes that rhabdomyoma of the heart is a simple embryonal tissue malformation, whose independent growth and complete development have been reached in embryonic time.

Steinbiss denies the necessity of looking for local disturbance factors to explain these tumors. In the presence of their marked association with brain, kidney and skin processes, and various congenital malformations, Steinbiss believes the underlying process must take place early before the differentiation of the germinal layers. He offers a simple, somewhat naïve explanation, which nevertheless has something appealing in its meaning. He regards the whole picture as a surplus formation — a “too much” in the heart anlage, a “too much” in the brain anlage, kidney anlage, etc.

The evidence seems to point against a true neoplastic nature of this growth. At any rate, it appears unsatisfactory to call upon local mechanical disturbances to explain its origin. To put it into the hamartoma grouping and to leave its origin cloaked in the obscurity characteristic of so many deviations from the normal in growth, seems to dispose best of all this perplexing question in the rhabdomyoma literature.

Schmincke's case<sup>2</sup> deserves special mention, since it was the only one of its type in this series. In a newborn infant, he found a heart weighing 46 gm., with measurements of 1.3 cm. and 1 cm. for the right and left ventricular walls respectively. The entire myocardium consisted of rhabdomyoma cells. No mature myocardial cells were found. In the hypertrophied papillary muscles, differentiated cells were present. Uehlinger's case presented certain similar features but contained definite nodules as well. In 1896 Virchow<sup>40</sup> expressed the theory that “idiopathic” hypertrophy of the heart could be explained by a diffuse rhabdomyoma of the myocardium but he lacked a case to demonstrate this. Schmincke, in his report, resurrected the old Virchow theory by illustrating it with his case, and came to the conclusion that diffuse rhabdomyoma of the heart explained the “idiopathic” hypertrophy. We have been able to study several hearts in cases of “idiopathic” hypertrophy and in no case could we demonstrate the presence of rhabdomyoma.

Only a brief discussion of the frequently associated cerebral tu-

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## DESCRIPTION OF PLATES

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### PLATE 23

FIG. 1. Heart with right auricle exposed. Large tumor mass obstructs tricuspid valve orifice.

FIG. 2. Heart with right auricle and right ventricle exposed. Note extension of large tumor mass from auricle into ventricle. Many small tumor nodules can be seen on posterior wall of right ventricle.

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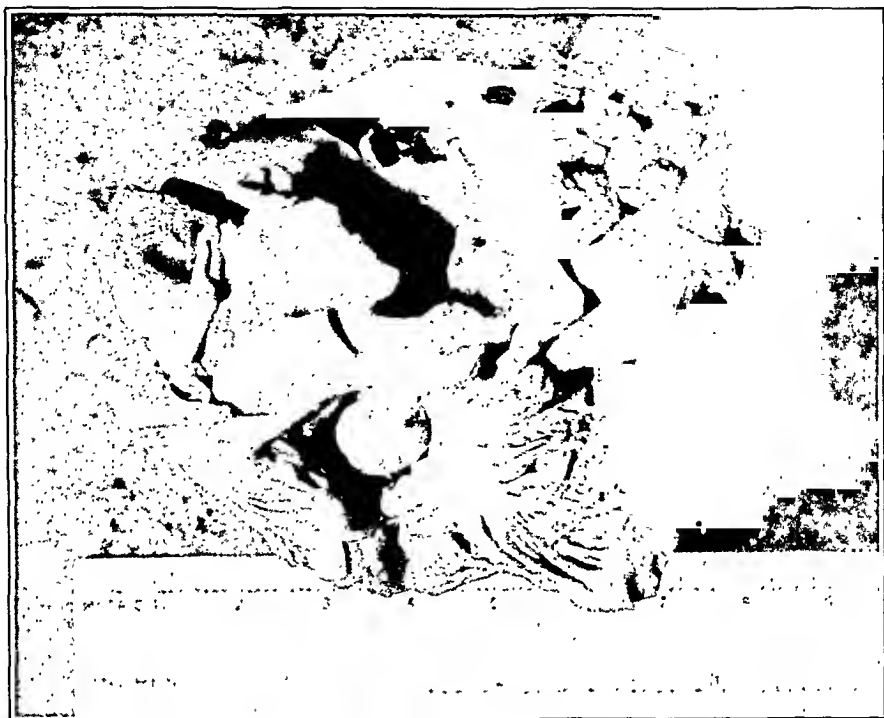
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PLATE 24

FIG. 3. Photomicrograph of tumor nodule. Phosphotungstic acid hematoxylin stain. Typical view of architecture of tumor. Note "spider" cells and large spaces.  $\times 200$ .

FIG. 4. Photomicrograph of tumor. Phosphotungstic acid hematoxylin. Shows large cell with cross-striations in fibrils and at periphery.  $\times 1600$ .



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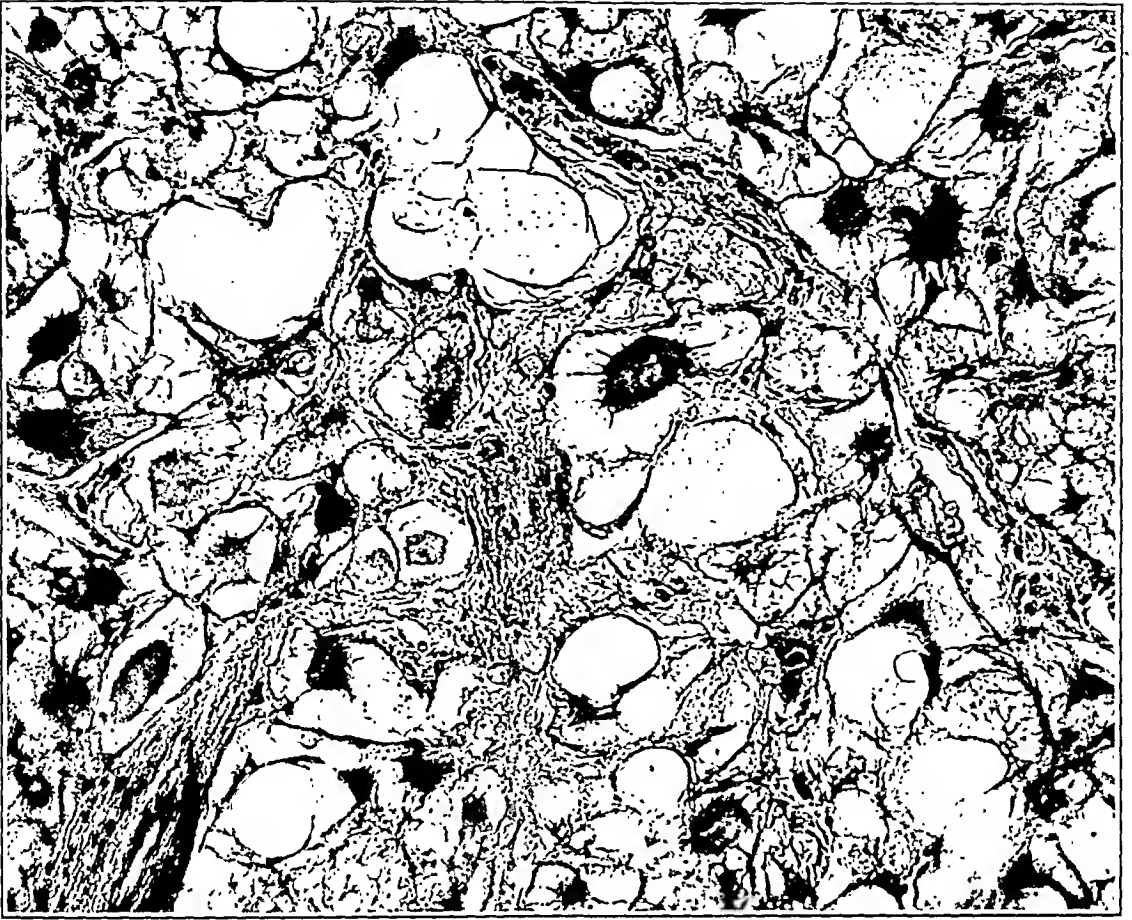


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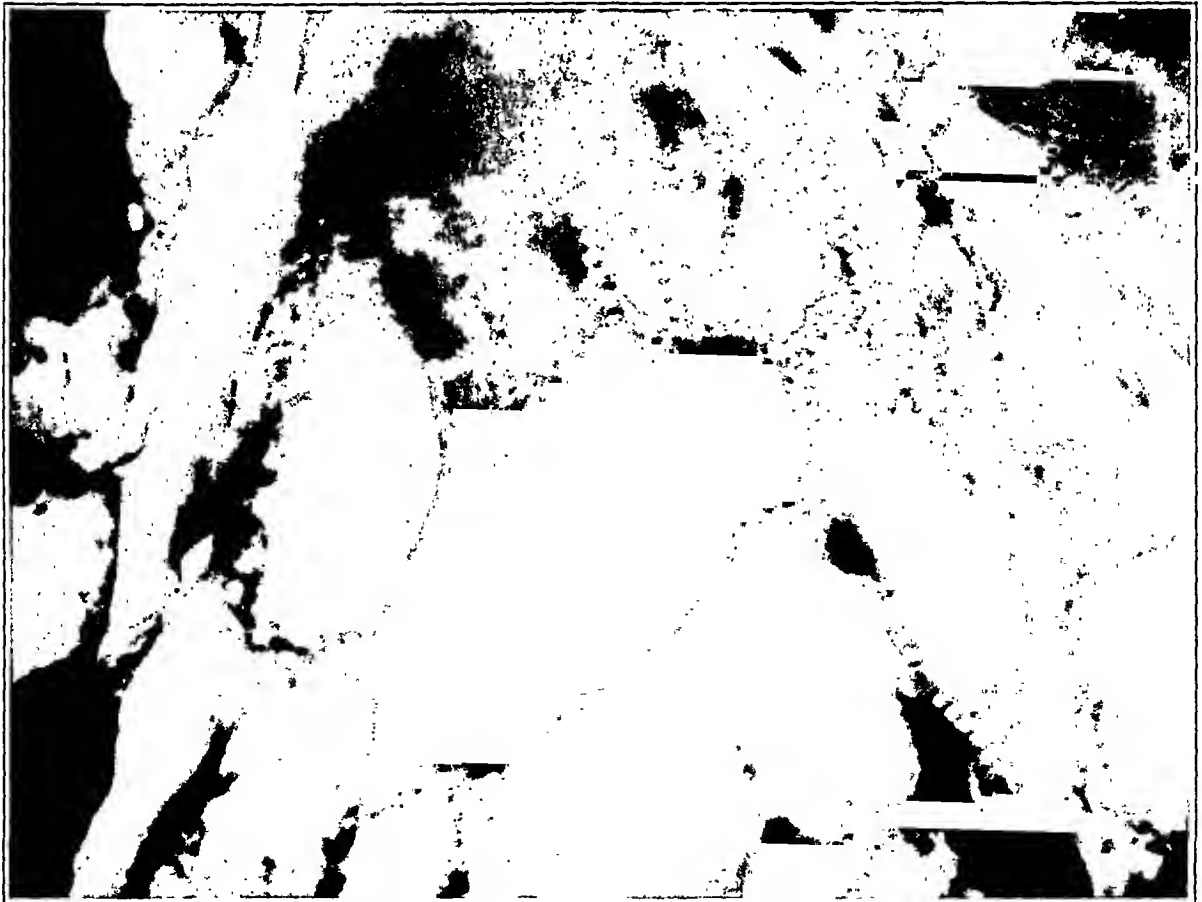
PLATE 25

FIG. 5. Photomicrograph from grossly normal myocardium. Phosphotungstic acid hematoxylin stain. Shows isolated collection of tumor cells with empty spaces.  $\times 200$ .

FIG. 6. Photomicrograph from tumor nodule. Best's carmine stain. Note large cell with large numbers of glycogen droplets.  $\times 850$ .



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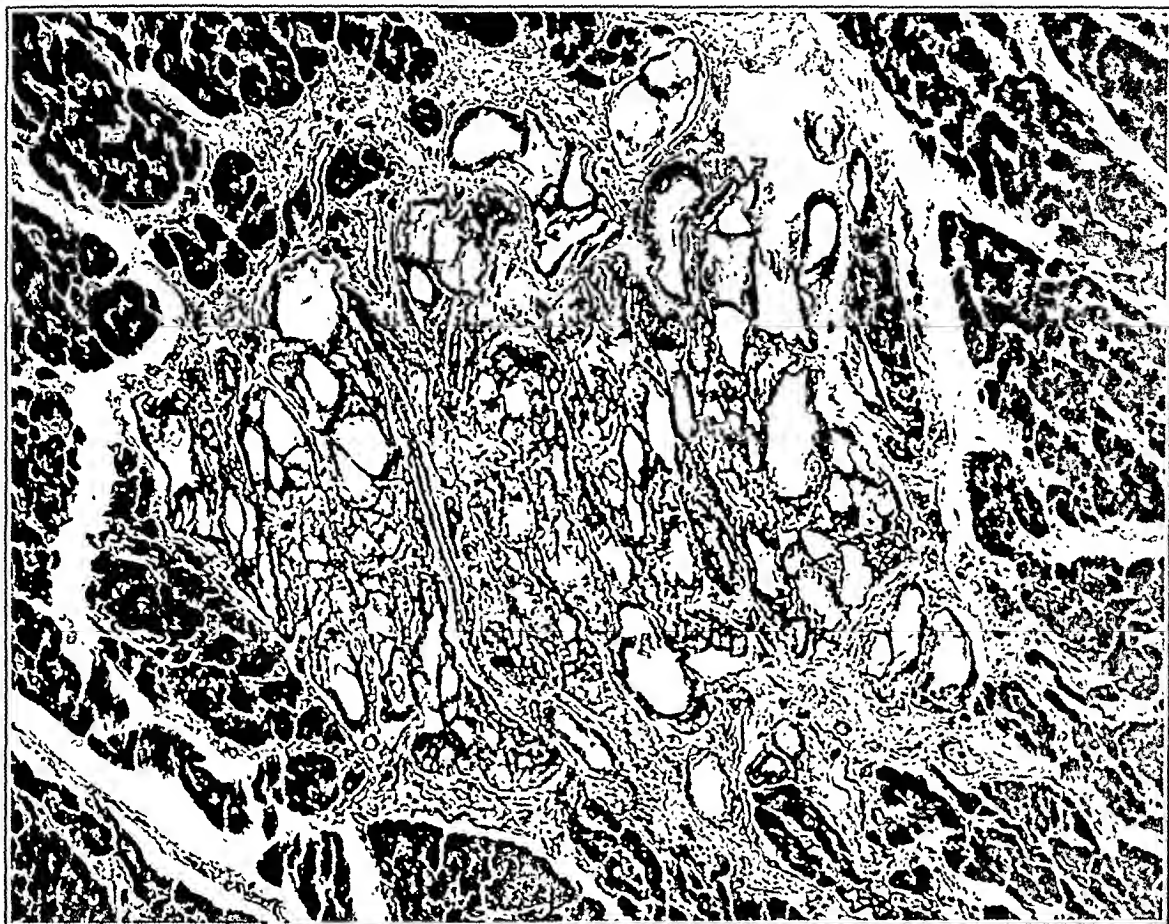
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PLATE 26

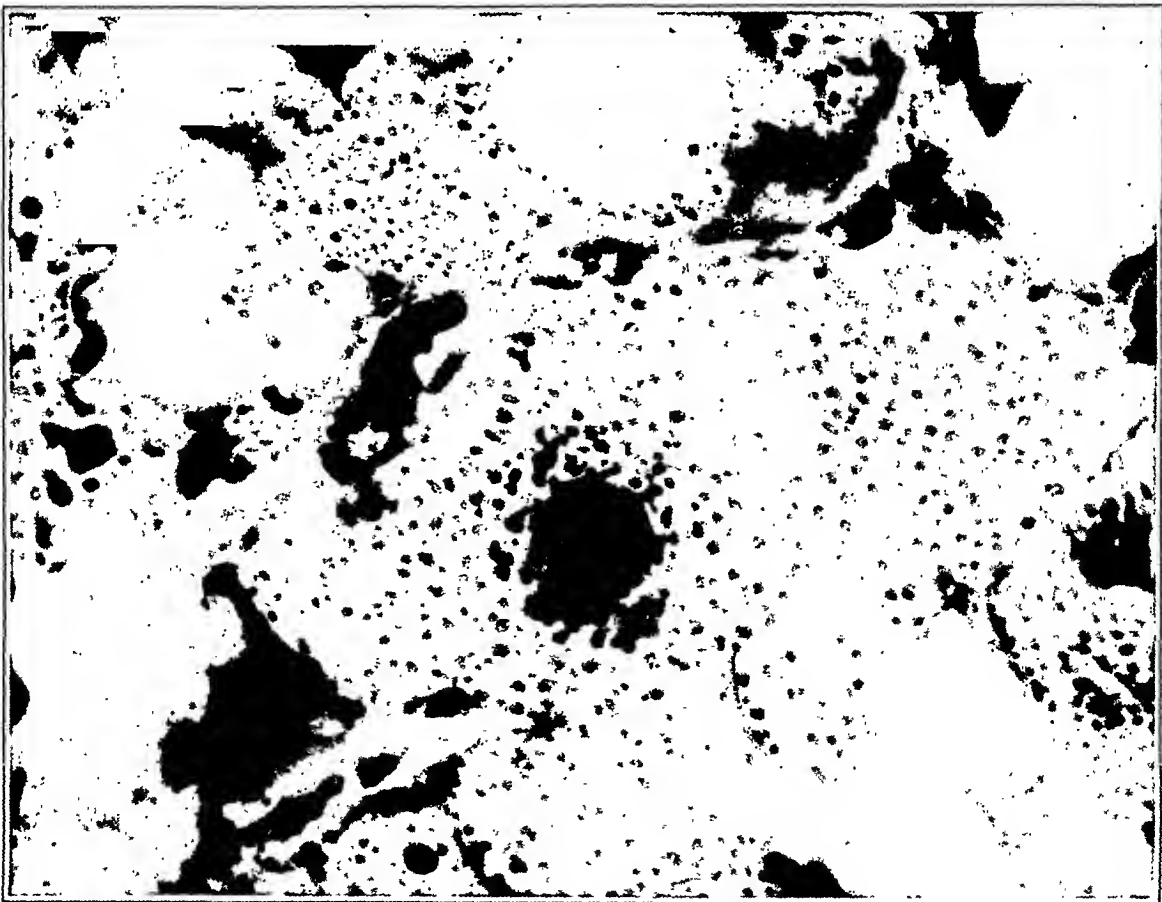
FIG. 7. Kidney with several cysts.

FIG. 8. Brain showing convexity. Shaded areas represent distribution of the tuberous sclerosis.

FIG. 9. Brain, lateral view, showing distribution of tuberous sclerosis.

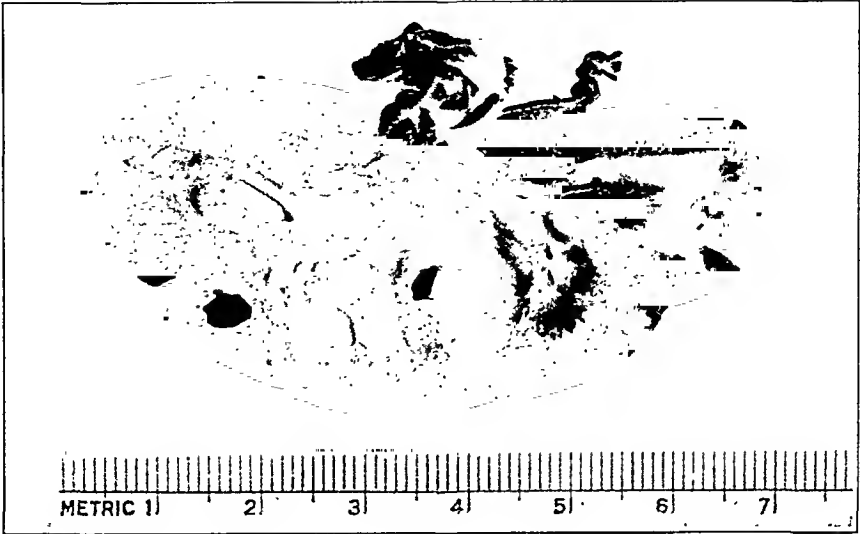


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## TECHNICAL METHODS

The directions given by Foot <sup>4</sup> were followed in the preparation of the silver ammonium carbonate. Full-grown rats were etherized and bled from the heart. About 10 cc. of the silver solution were injected into the portal vein to free the liver of excess blood and finally the solution was injected directly into the liver until it lost color and assumed a translucent appearance. The solution was injected directly into the substance of the spleen until it was distended to twice its normal thickness and until the tissue became white at points of greatest concentration. The lymph nodes situated near the aortic bifurcation were distended with the solution. After 20 to 30 minutes, the spleen, lymph nodes and liver were fixed in 10 per cent formalin. Paraffin sections prepared in the usual way were stained with hematoxylin and eosin. The reduced silver is not affected by the usual staining methods, but any excess of dye obscures the yellowish silver granules. The best results have been obtained by staining lightly, about 10 to 15 seconds in Harris' hematoxylin without acetic acid, and in dilute eosin about 10 seconds. As in the use of neutral red it was found that the silver solution kills the cells at once where it reaches them in greatest concentration, but that in adjoining territory where its strength is reduced by contact with the dead tissue and by diffusion the supravital effect is produced. In addition to the full strength solution all dilutions, 1:2, 1:3 and so on to 1:10, have been injected into the organs of numerous rats. Dilutions above 1:4 are usually ineffective. A 1:2 dilution with distilled water, or the undiluted solution is the best for all tissues. In the dead tissue areas the silver staining is diffuse and has extended to nuclei and intercellular substance. The yellow color of the nuclei becomes obscure after application of the hematoxylin. Such areas must be avoided in a study of the vital reactions.

## HISTOLOGICAL FINDINGS

*Spleen:* Distention of the organ by the silver solution and its fixation in the distended state makes conspicuous the structural relations described by Robinson.<sup>5</sup> The solution does not penetrate the malpighian corpuscles well, and attention has been directed especially to the venous side of the circulation. The veins leave the capsule and trabeculae, lose their connective tissue support and pass

## SUPRAVITAL STAINING WITH SILVER AMMONIUM CARBONATE \*

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Since impregnation with silver makes visible in a remarkable way structures otherwise not well seen, it has obvious value. However, scarcely any other substance colors such a wide variety of related and unrelated cellular products as does silver. In the connective tissue group especially fine shades of distinction have been attempted with the silver impregnations and many conflicting views have consequently arisen. The application of the various methods has been almost exclusively to dead tissue, and with few exceptions to dead fixed tissue. The outstanding exception is the method of Taft and Ludlum,<sup>1</sup> in which they treated unfixed brain with argyrol. In their preliminary report they state that they used fresh tissue from experimental animals. The first experiments made in the present investigation were with argyrol which was injected directly into living tissues by a method previously used to obtain supravital staining with neutral red.<sup>2</sup> The phagocytes were found to react as they do to carbon in fine suspension<sup>3</sup> and not as they do to neutral red. Silver ammonium carbonate was substituted for the argyrol and found to penetrate the cytoplasm of certain living cells as readily as the dyes used in the supravital stains. Once within the cells the silver was reduced and precipitated in the usual way, where it remained permanently. The chief handicap of supravital staining with neutral red has been inability to retain the dye without change in sections of the embedded tissue. The author<sup>2</sup> published the method for mordanting the neutral red with chrome salts. Later several methods using chrome salts as a mordant were published by others, but all were of limited value on account of solution of much of the dye during the embedding and staining processes. The method had some value since a little of the dye was retained in the sections of tissue and some of the cellular relationships could be distinguished.

\* Received for publication December 31, 1930.

cytoplasmic processes, often two in number, that connect them (Fig. 5). These cells are much like the phagocytes that grow in tissue cultures of lymph nodes.<sup>6</sup> Free in the sinuses, there are many large round cells that have separated after the injection and before fixation. In both attached and detached cells the cytoplasm contains large numbers of silver granules, regular in size and shape. In most cells the granules are scattered throughout the cytoplasm, but in some they are clumped in one mass. The silver preparations show to be true what was little more than surmised in the tissues treated with neutral red. The reacting cells are not confined to the sinuses but extend into the dense follicles where groups of non-reacting lymphocytes lie in their interstices. In this dense tissue the silver granules are usually in a single group and not infrequently a clear space appears at the center of the mass. The silver-reacting cells in the sinuses connect with those in the follicles. Both are very different in appearance from the histiocytes of the spleen. The cytoplasm is dense and the cell outline perfectly distinct. The silver granules are spherical and of uniform size. The cells readily round up and separate. The nuclei are relatively large and not infrequently cupped at one side (Figs. 2 and 3). The narrow flat cells of the sinus walls show no reaction in their scant cytoplasm.

### DISCUSSION

Any investigation of free cells should take into account their origin from a fixed tissue. A prevalent view in regard to free mononuclear phagocytes is that they do not arise from a fixed tissue but that they exist instead as a distinctive cell type that separates during embryonic development to persist in various organs and tissues throughout life as wandering or wandering "resting" cells. Maximow<sup>7</sup> was of the opinion that one fraction of the mononuclear phagocytes was of this type and that the other was derived from lymphocytes by a phagocytic transformation. Certainly most free cellular elements such as lymphocytes, granular leukocytes, red blood corpuscles and blood platelets are fixed-tissue derivatives and the author<sup>3</sup> has for many years adhered to the view that the mononuclear phagocytes of the higher vertebrates have a base of supply in the fixed tissues.

The observations made on the supravital silver stains strengthen the view previously expressed by the author,<sup>8, 9</sup> which gave recogni-

into the lobules as capillaries consisting of a single layer of endothelium. Often the terminus of the capillary can be distinguished. Usually the lumen widens and the narrow endothelium flares out into a network of large reticular cells. The size of the lumen depends on its distention by the injected fluid and not infrequently the two rows of endothelial cells terminate with the network of silver-staining cells beyond. The terminal expansions may be lined on one side with flat cells and on the other with reticular cells (Fig. 4). Near the splenic capsule the injection is best. The histiocytes are made conspicuous by the yellow granulation. Without the silver the cytoplasm of these cells stains lightly and the cell outline is indistinct. Where the histiocytes are spread apart they are seen to be connected by innumerable fine fibrils which do not react to the supravital silver but which take the stain in the areas of dead tissue. The silver granules are irregular in shape, size and distribution. In most of the cells the granules are scattered throughout, but in some there is a mass of granules occupying one segment of the cell. The nuclei of the histiocytes are relatively small and have some tendency to an oval shape. The flat endothelial cells contain no silver. In most of the capillaries there is not much evidence of the flat cells undergoing a gradual transition to form the larger histiocytes.

*Liver:* The injection of the liver is not as satisfactory as that of the spleen. The sinusoidal endothelium is pressed against the hepatic cells. Also, so much of the silver is absorbed by the abundant parenchyma that it is difficult to regulate the strength of the solution. However, in selected areas the Kupffer cells are distinct and in their cytoplasm is seen a variable number of irregular silver granules, unevenly distributed. There is little evidence of any massing of the granules about a centrosphere. The largest of this type of cells shows a delicate cytoplasm resembling the histiocytes of the spleen, but the network of fibrils about them and connecting them is not shown. This appears to be dependent on a stretching and spreading of the vascular network which does not follow the injections as it does in the spleen.

*Lymph Nodes:* In general structure and in function the reticular tissue of lymph nodes is unlike that of the spleen. Both these organs ingest particulate matter and segregate colloids, but in different fashion. In the medulla the cells of the sinuses are well separated by the force of the silver injection. Many of the cells reveal graceful

usual non-phagocytic type became large, phagocytic, and even assumed a branching reticular appearance when it was sufficiently stimulated (Fig. 1). Again, in observations on granulation tissue in the liver,<sup>11</sup> it was seen that the sinusoidal endothelium, normally phagocytic in this location, became inactive and non-phagocytic when contact with hepatic parenchyma was lost. In the spleen the structural relations indicate an identity of endothelium and reticular cells. The two are continuous and the histiocytes in part form the walls of the terminal capillaries. It is likely that the two are interchangeable in form and function. When the cell shrinks it becomes non-phagocytic and non-reactive to solutions of silver. Under normal conditions, the terminal or functional cells are phagocytic for coarse particulate matter such as senile or injured red blood corpuscles and fragmented lymphocytes. In the performance of their function of ridding the blood of undesirable particulate matter the histiocytes tend to remain anchored by the numerous fine fibrils that connect them to form the splenic pulp. They do not have a great tendency to round up and float free for mobilization at a distance. Such behavior indicates that their activity is largely local. The capacity of these cells to incorporate and segregate the silver salt is related to their selective activity for colloids. The argyrophilic sinusoidal endothelia or histiocytes made up of the terminal cells of the vascular system are, therefore, more than mere tubes for blood transportation. It is the exercise of these added functions that is responsible for the change from inactive flat cell to phagocytic histiocyte. The difference between flat endothelium and histiocyte appears less than the usual difference between duct epithelium and the secreting portions of glandular organs. The relationship, however, is somewhat analogous.

*Monocytic Tissue:* Evidence of the origin of monocytes from lymphoid tissue is overwhelming. Maximow<sup>7</sup> maintained that these cells constitute a lymphocyte-monocyte group with the lymphocytes on stimulation readily becoming monocytes. That the two may arise from a single stem cell is suggested by the extension of the argyrophilic cells from the sinuses into the follicles as described in connection with the lymph nodes. These solid cords of reacting monocytic cells may to some extent represent potential lymph channels, but it must be kept in mind that their proximity to the medulla may be explained by the successful penetration of this tissue by the

tion to essential differences between the so-called reticular cells of lymphoid tissue and reticular cells of sinusoidal organs such as the spleen and liver. Although the functions performed by these two groups are so very different they are quite generally classed together as the reticulo-endothelial system. Sabin, Doan and Cunningham<sup>10</sup> in the examination of splenic puncture material in the supravital films found a rosette type and a diffuse-granule type of cell. Without an investigation of the *in vivo* relationship of the reacting cells they concluded that the rosette form was derived from reticular tissue in the spleen and other sinusoidal organs but not from the lymph nodes, since they found few of the rosette forms in the lymph gland punctures. The diffuse-granule cell was thought by them to be derived from capillary endothelium. The evidence now is that the arrangement of supravital granules within the mononuclear phagocyte depends on the age and functional activity of the cell rather than on its kind.

*Histiocytic Tissue:* The nomenclature used in connection with the mononuclear phagocytes is burdensome, and it is desirable not to make it more so. Fortunately, the mononuclear cells present in the peripheral blood are almost universally called monocytes. Tissue from which they arise may well be called monocytic tissue. To exclude a misunderstanding, histiocytic tissue is, in this article, applied to the sinusoidal endothelial network with the implication that some cells may separate from it to become free histiocytes. The term has, of course, been used in a much broader sense. The view that the vascular network may, during embryonic development, arise *in situ* from the mesenchyme is well supported, but there is less evidence that endothelium may differentiate from a connective tissue base in the fully matured organism. There are two main explanations for the characteristic vascular network of sinusoidal organs. Maximow maintained that it was unrelated to endothelium and called the cells composing it histiocytes. This implies that in several organs the blood passes from its endothelial-lined channels to enter spaces lined by an unrelated type of cell. The alternative view is that endothelium as a mesenchymal derivative may occur in either of two forms. The difference between ordinary flat endothelium and reticular cells is not necessarily interpreted as due to a difference in cell type. In granulation tissue experimentally produced beneath skin surfaces the author<sup>8</sup> found that the capillary endothelium of the

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## DESCRIPTION OF PLATE

## PLATE 27

- FIG. 1. Capillary in subcutaneous granulation following large injections of trypan blue, and finally, intravenous injection of India ink. The phagocytic cells containing ink particles are structurally like the histiocytes of the spleen.
- FIG. 2. Lymph node. Free monocytic cells within sinuses. The two lymphocytes show no silver granulation.
- FIG. 3. Lymph node. A small group consisting of two silver-reacting monocytic cells and two lymphocytes outside the sinuses.
- FIG. 4. Spleen. Terminal capillary with histiocytic silver-reacting cells on one side.
- FIG. 5. Lymph node. Monocytic silver-reacting cells of medullary sinuses.

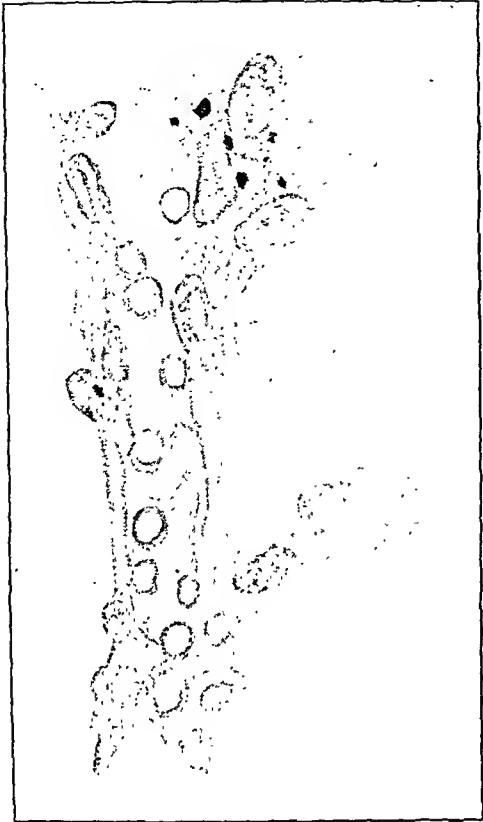
injected fluid. Naturally, the silver does not penetrate the solid tissue readily, and as a result the entire distribution of the monocytic tissue has not been determined. The fixed monocytic cells outside the sinuses are rounded, and within the sinuses, elongated. In the former the argyrophilic granules are often grouped into a single mass, while the cells within the sinuses usually show more than one group of granules, or frequently the cells present a diffuse granulation. Great numbers of the cells round up and separate after the injections are made. These free cells have all the characters of the blood monocytes. The monocytic tissue plays a dominant rôle in diseases such as typhoid fever and exudative tuberculosis. It is readily stimulated to form large epithelioid cells with great masses of supravital granules and to participate in the formation of foreign body giant cells. As a circulating leukocyte, the monocyte appears in inflammatory foci almost as quickly as does the granular leukocyte. Although it may undergo mitosis in the lesions, it is recruited chiefly from the lymphoid tissue where the quantity of monocytic tissue almost equals that of the lymphocytic.

### CONCLUSIONS

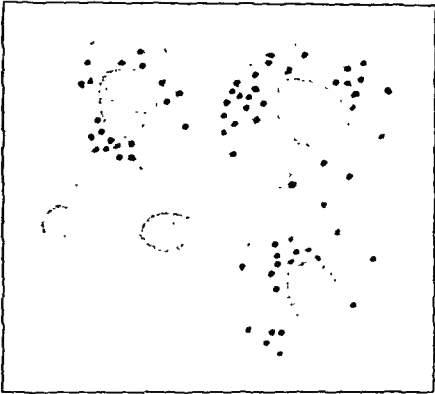
1. Silver ammonium carbonate may be used to mark living cells supravitally.
2. By supravital staining with silver ammonium carbonate the origin of the monocytes is seen to be from the silver-marked portion of lymphoid tissue.
3. The histiocytic tissue of the sinusoidal organs also reacts to supravital silver but the response is unlike that of the monocytes.
4. The two component parts of the so-called reticulo-endothelial system are so unlike in function and structure that they should not be grouped together. The histiocytic tissue of the sinusoidal organs consisting mostly of anchored cells functions chiefly as a fixed tissue. On the other hand, the monocytes originating in the lymphoid tissue are a normal element of the circulating blood and may be concentrated quickly in any tissue or cavity.



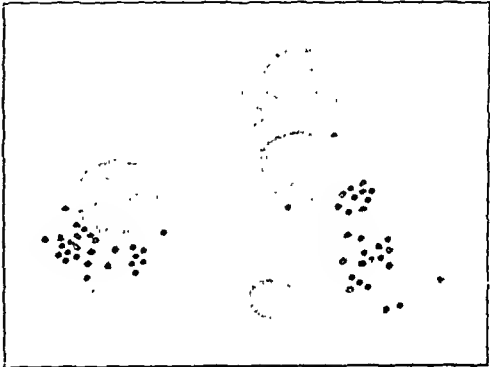




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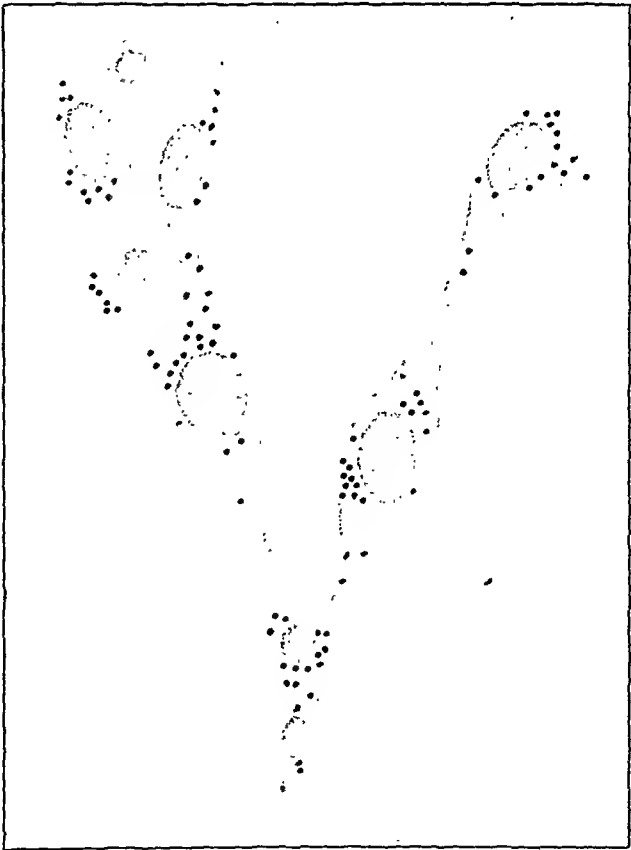


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Supravital Staining with Silver

flesh and without gross evidence of disease. Microscopic examination disclosed lesions of a tuberculous character in the liver and spleen, but the lungs and kidneys escaped demonstrable involvement.

LOT 2: Injection was made with *Mycobacterium tuberculosis* of avian origin. Gopher 6, which had been given an injection intravenously, died approximately three hours after injection. Gopher 7, which was given an injection intravenously, was killed for autopsy after sixty-six days. Grossly the only lesions were multiple, minute grayish foci in the liver. Many tuberculous lesions were present in the liver and spleen, but in the lungs and kidneys lesions were not found. Cultures were obtained from an emulsified portion of the liver, and a chicken that was inoculated intravenously with 1 cc. of the same emulsion was found to have tuberculous lesions when killed for autopsy sixty-seven days after the inoculation. A guinea pig, when given an injection with a portion of the liver emulsion of Gopher 7, failed to reveal lesions when killed for autopsy after sixty-one days. Gopher 5, which had been inoculated subcutaneously, was killed for autopsy after ninety-one days, and lesions were not found on gross or microscopic examination. Gopher 8, which had been inoculated intraperitoneally, was also killed for autopsy after ninety-one days and was without discernible alterations in either the lungs or the kidneys, but a few definitely tuberculous lesions were present in the liver and spleen.

LOT 3: Injection was made with *Mycobacterium tuberculosis* of human origin. One of the animals which had been inoculated intravenously, Gopher 10, died forty-five days after the injection. Before the animal was found, all the viscera had been eaten by the other animals in the cage, and no suitable material remained for study. Gopher 9, which was inoculated intravenously, died in forty days and numerous and extensive tuberculous lesions were visible grossly throughout the liver, spleen and lungs. A few small tuberculous foci were present in the kidneys. The lesions were most numerous in the medullary portion of the organ. Gopher 12, which had been inoculated subcutaneously, was without demonstrable lesions when killed for autopsy sixty-six days afterward. Gopher 11 died seventy-eight days after receiving an intraperitoneal injection of the bacterial suspension. At autopsy extensive lesions of tuberculosis were observed throughout both lungs (Fig. 1). A few nodular lesions

# SUSCEPTIBILITY OF THE GOPHER (*CITELLUS TRIDECEMPLINEATUS*) TO *MYCOBACTERIUM TUBERCULOSIS* \*

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In order to determine the anatomical reaction of the thirteen-lined ground squirrel, *Citellus tridecemlineatus* (Mitchell) to the three bacillary types of *Mycobacterium tuberculosis* the following experiment was performed.

Twelve adult animals were captured in their natural haunts and divided into three lots, each containing four animals. The animals in each lot were inoculated with 0.5 cc. bacterial suspensions prepared from 48-day-old cultures of *Mycobacterium tuberculosis* as follows: two intravenously, one subcutaneously, and one intraperitoneally.

The organisms used were original strains of *Mycobacterium tuberculosis* which had been isolated from human, bovine and avian sources and each had been proved by tests of pathogenicity with guinea pigs, rabbits and chickens to be true to type. After being inoculated, each lot of four animals was placed in a separate cage for observation.

## RESULTS

LOT 1: Injection was made with *Mycobacterium tuberculosis* of bovine origin. About six weeks subsequent to the injection two of the animals (Gophers 2 and 3) escaped at feeding time and successfully avoided recapture. One had been inoculated intravenously and the other intraperitoneally. Of the two remaining animals the one which had been inoculated intravenously (Gopher 1) died after thirty-six days. The lungs had been eaten by cage mates, but the abdominal viscera were intact. The spleen was slightly enlarged and contained a few grayish white foci. Microscopic examination disclosed numerous tuberculous lesions throughout the liver and spleen, but lesions were not observed in the kidney. Gopher 4, which had been inoculated subcutaneously, appeared weak after a lapse of forty days, and was killed for autopsy. The animal was in good

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cells observed were those in the splenic lesions of the animal which had been inoculated intravenously (Gopher 7, Fig. 3). The lesions in this instance had their inception within the splenic corpuscles but were limited to one area near the periphery. In no instance were the lesions observed to occupy the central portion of this structure. In the spleen of Gopher 8, which had been inoculated intraperitoneally, the lesions consisted almost entirely of large multinucleated giant cells, often in clusters of three to five. Although these structures were identical in appearance to those definitely associated with true tuberculous lesions, acid-fast bacteria could not be demonstrated within them.

The liver of Gopher 7, which had been inoculated intravenously, contained a large number of focal lesions, and acid-fast organisms were numerous in each. Neither encapsulation nor calcification was observed.

By far the most extensive lesions of tuberculosis occurred in those animals which received *Mycobacterium tuberculosis* of human origin. The extent and character of the lesions, however, appeared to be quite dependent on the route of inoculation, for although the lesions were exceedingly extensive in the animals which had been given injections intravenously, none could be found in the tissues of the one inoculated subcutaneously. The animal that was inoculated intraperitoneally, although possessing a great number of lesions, many of which were extensive, did not have the widespread malignant type of lesions which were so noticeable as a consequence of the intravenous exposure. In the animal inoculated intravenously the lesions of the liver and spleen, which had replaced much of the normal tissue of these organs, were diffuse, and many of the monocyctic cells had undergone coagulation necrosis. Epithelioid cells were not apparent. In those lesions in which the cellular integrity remained intact the monocyte of the early tubercle was the cell which was present.

Giant cells, some of which were very large, were exceedingly numerous in the hepatic and splenic lesions where they were associated with the collection of monocyctic cells making up the lesion (Fig. 4). Acid-fast bacteria were present in large numbers in all of the lesions of the liver and spleen.

The lungs of the gophers that were inoculated with the organism of human origin intravenously and intraperitoneally were involved

were also present in the serosa of the intestines. Although the spleen was perhaps slightly enlarged, definite lesions were not seen. Grossly, the liver and kidneys appeared normal. Microscopically there were numerous and extensive lesions of a tuberculous character in the spleen and the liver, but the kidneys were not involved.

### HISTOPATHOLOGY

From all the tissues obtained for histopathological study two series of sections were prepared. One series was stained with the usual hematoxylin-eosin combination and the other was stained with carbol fuchsin-hematoxylin for the purpose of staining acid-fast bacteria which might be present. Considering first the tissues obtained from the animals in which there were established lesions of tuberculosis following the injection of organisms of bovine origin, the most extensive lesions were found in the tissues of the animal inoculated intravenously. Both the liver and spleen contained many spherical collections of monocyctic cells among which were large numbers of acid-fast bacteria. Very few of the monocytes had assumed an epithelioid appearance, although caseation necrosis was beginning in the center of many of the collections. Encapsulation or calcification was not seen. Associated with most of the lesions, particularly the smaller or earlier ones, were characteristic giant cells. These cells were of two general varieties. In one the nuclei were arranged at the periphery of the cytoplasmic mass and in the other the nuclei were grouped more or less centrally. Each of the giant cells possessed large numbers of acid-fast organisms. The lesions which were limited to the liver and spleen failed to show any particular predilection for a specific portion of the respective organs, except it was noted that in the spleen of the animal which had been inoculated subcutaneously, the lesions were in the splenic pulp and not within the splenic corpuscles.

Except for those lesions which occurred in the animal inoculated intravenously, the lesions that resulted as a consequence of the organisms of avian origin were much fewer than those which occurred following the injection of the mammalian strains of *Mycobacterium tuberculosis*. They were, however, essentially of the same character, consisting of collections of monocyctic cells with a tendency to undergo caseation necrosis (Fig. 2). The only definite epithelioid

From the results of my studies it is apparent that there exists a difference in the susceptibility of the striped gopher to the three forms of *Mycobacterium tuberculosis*. The difference in the resistance to each of the three forms of the organism seems to be relative and dependent to some degree on the route of inoculation. In the animals which were given injections intravenously, well defined lesions of tuberculosis developed, regardless of the source of origin of the infecting bacteria. However, the organisms of avian origin proved less successful in inciting lesions when they were introduced subcutaneously and intraperitoneally. Although some of the animals that were injected with the human and bovine forms of the organism died spontaneously within thirty-six to forty days after the injection, those that received organisms of avian origin lived until they were killed for autopsy sixty-six to ninety days after inoculation. This indicates the possession by the gopher of a greater resistance to the avian form of the organism than to either the human or bovine form. The resistance of the gopher to the avian form of *Mycobacterium tuberculosis* was further evident from the fact that even though numerous lesions occurred in the liver of the animal injected intravenously with this organism, the lungs were without demonstrable lesions. This suggests that the lungs of the gopher possess a peculiarly efficient resistance to the avian form of *Mycobacterium tuberculosis* similar to that observed in the lungs of the dog.<sup>2</sup>

The cellular reaction to the organism of tuberculosis in each of the animals was essentially the same, regardless of its source of origin. Any differences noted were largely differences in the extent of the lesions rather than in their fundamental histological character. The unusually large giant cells which were so numerous in association with many of the cellular reactions would seem to be of some significance in the attempt on the part of the tissues to establish and maintain a protective mechanism against the bacteria of tuberculosis. The apparent absence of these cells in the pulmonary lesions, however, is difficult to explain. Perhaps their formation is an index to the degree of resistance exhibited by the monocyctic cells of the invaded tissues to the infecting organisms, since the pulmonary lesions in the tissues studied showed very little, if any, arrestment of the disease.

The failure to observe lesions of tuberculosis in Gopher 12, which had been inoculated subcutaneously with the bacterial suspension

in a most intensive tuberculous process (Fig. 1). Although many tubercles were present, many of which were conglomerate, a cellular reaction indicative of excessive tuberculous infection was apparent throughout the entire substance of the organ. One of the lungs contained numerous large tuberculous abscesses consisting of caseated cellular débris surrounded by a rather wide, fibrous capsule. Enormous numbers of acid-fast bacteria were present, particularly in the peripheral zone of each of the abscesses. Many of the bronchi and bronchioles of the lungs of the animal inoculated intravenously were practically occluded by an exudative substance which contained acid-fast bacteria in large numbers.

Giant cells, which were so numerous in the liver and spleen of the gophers that were inoculated with the organisms of human source, were singularly absent in the lung tissue observed. Likewise calcification was not seen.

#### COMMENT

A review of the literature indicates that the common striped gopher has not heretofore been used to any extent in experimental tuberculosis. One reference only was found and this pertained to the use of this animal as a substitute for the guinea pig as a diagnostic aid in obscure tuberculous infections. The report referred to was by Hewetson,<sup>1</sup> a physician located at the time (1905) at Pincher Creek, Alberta, Canada. He found it difficult to obtain guinea pigs, and the prevalence of the gophers in that locality suggested the possibility of using the latter animal in the diagnosis of tuberculosis. He gave each of nine gophers an injection with 2 cc. of a fluid containing tuberculous sputum; in the animals that died thirty to forty days afterward, enlargement of the spleen was noted, together with lesions of a tuberculous character in this organ and in the liver. Lesions were not observed in the lungs. Hewetson concluded that the susceptibility of the gopher to tuberculosis was established and that those living in regions where these animals were numerous might use them in the laboratory diagnosis of tuberculous infections of an obscure character.

Hewetson failed to designate specifically the genus and species of the animals in his experiment, but since the striped gopher is said to be prevalent in that part of Canada in which his observations were made it seems reasonable to assume that some species of *Citellus* was used.



animals are more susceptible to organisms of bovine and human origin than to those derived from avian sources. The route of inoculation has a significant bearing on the induction of subsequent lesions, the intravenous route being the most effective.

### REFERENCES

1. Hewetson, S. W. The gopher: a possible substitute for the guinea-pig. A preliminary report. *Med. News*, 1905, 87, 824.
  2. Feldman, W. H. The pathogenicity for dogs of bacilli of avian tuberculosis. *J. Am. Vet. M. A.*, 1930, 76, 399.
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### DESCRIPTION OF PLATES

#### PLATE 28

- FIG. 1. Lungs of Gopher 11. Extensive tuberculous involvement. The animal died seventy-eight days after receiving intraperitoneally organisms of tuberculosis of human origin.
- FIG. 2. Tuberculous foci in the liver of Gopher 7. The animal was killed sixty-six days after the intravenous injection of bacteria of avian origin.  $\times 180$ .

of human origin, would make one hesitate to accept the conclusion that the striped gopher might be used as a satisfactory substitute for the guinea pig in determining obscure tuberculous infections. The animal in question received a dose of virulent organisms many times greater than would be likely in material obtained from clinical sources. Yet after a lapse of sixty-six days demonstrable lesions failed to develop. The number of animals used in this experiment constitutes a rather small group from which to draw definite conclusions, but the absence of lesions in Gopher 12 seems of sufficient significance to question the reliability of the use of the gopher as a routine diagnostic aid in determining tuberculous infections. Although infection would probably follow in every instance if sufficient virulent organisms were introduced intravenously, for technical reasons this procedure is not a practical one so far as the gopher is concerned.

#### SUMMARY AND CONCLUSIONS

1. With the use of original strains of *Mycobacterium tuberculosis* of human, bovine, and avian origin, a series of gophers (*Citellus tridecemlineatus*) was inoculated to determine the susceptibility of this animal to the respective bacillary forms of the organism of tuberculosis. With each organism two animals were injected intravenously, one subcutaneously, and one intraperitoneally. Two of the twelve animals injected escaped after being under observation for six weeks and were not recaptured, and one animal died three hours subsequent to injection. Of the remaining nine gophers, four died after intervals of from thirty-six to seventy-eight days, and the others were killed for autopsy at from forty to ninety-one days after the injection.

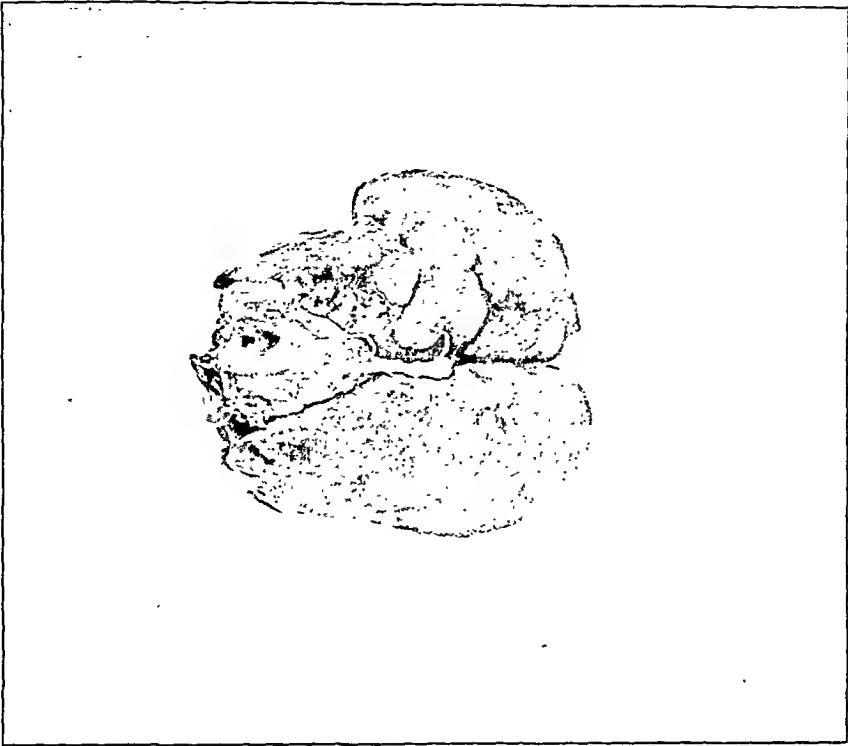
2. Definite lesions of tuberculosis were observed as a consequence of each of the three forms of *Mycobacterium tuberculosis*, with the degree or extent of the infection varying with the route of inoculation, being most pronounced in the animals that were inoculated intravenously. A histopathological study of the lesions in each of the animals was made.

3. By experimental methods it is possible to induce lesions of tuberculosis in the striped gopher (*Citellus tridecemlineatus*) with each of the three bacillary forms of *Mycobacterium tuberculosis*. The

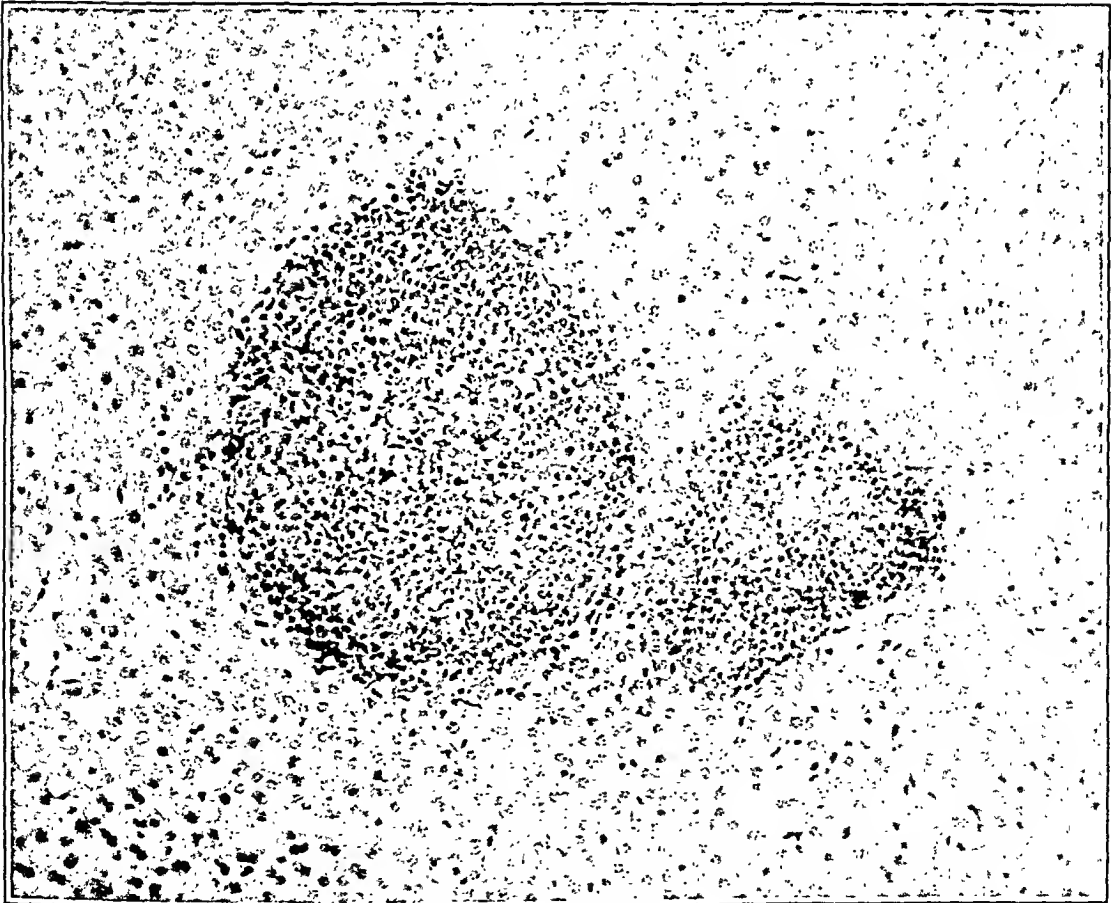
PLATE 29

FIG. 3. Collection of epithelioid cells replacing the normal elements of a splenic nodule of Gopher 7. The animal was killed sixty-six days after receiving an intravenous injection of bacteria of avian tuberculosis.  $\times 150$ .

FIG. 4. Large tuberculous giant cell containing many acid-fast bacteria, from a hepatic lesion in Gopher 11. The animal died seventy-eight days subsequent to the intraperitoneal injection of *Mycobacterium tuberculosis* of human origin.  $\times 1100$ .



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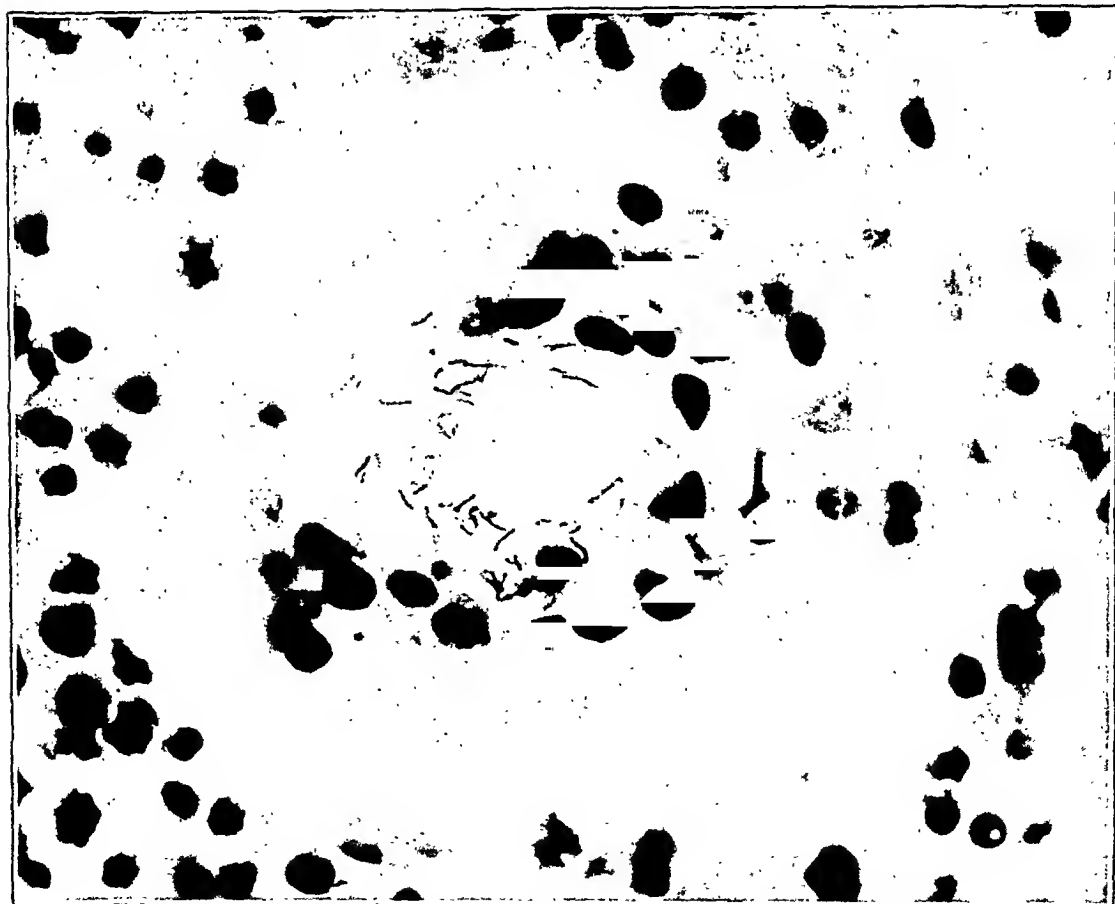


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year after injection. In only one of the animals were lesions demonstrated which could be considered of a definitely tuberculous nature. In three others, however, a few lesions were observed which were histologically identical with those of tuberculosis, although acid-fast organisms were not found among the cells constituting the respective lesions. The lesions in three of these four animals were confined to the liver and were of microscopic dimensions. With one possible exception, in none of the ten animals were lesions found grossly or microscopically in the lungs, spleen or kidneys.

Histologically the lesions in the liver of the animals were essentially the same after intravenous injections. They consisted of irregular, ovoid to spherical masses of monocyctic cells, distributed among which were a few cells of a lymphoid character (Fig. 1). The larger masses occurred in the connective tissue elements surrounding the portal canals, whereas the smaller foci were present among the cells of several of the lobules of the liver. None of the separate lesions was in any sense large, and although the lesions were sharply separated from the adjacent parenchymatous cells, they were not divided from them by interposing elements. The zone of contact was devoid of a definite cellular response which might be interpreted as an expression of resistance. The Langhans' type of giant cell was not apparent in any of the material from the liver, nor were definite necrobiotic changes seen. Although the lesions in the liver of three of the dogs failed to reveal acid-fast organisms by appropriate staining methods, organisms morphologically indistinguishable from *Mycobacterium tuberculosis* were demonstrated in the fourth animal of the series. In addition, the organisms were successfully cultivated from the liver by the method of Corper and Uyei,<sup>2,3</sup> and typical lesions of tuberculosis developed in a chicken following intravenous injection with a portion of an emulsion prepared from the liver of this dog. The lesions in this liver were much more numerous than in the liver of the other three dogs and they appeared to be of a progressive nature, in contradistinction to the more or less quiescent appearance of the others.

In some of the sections prepared from the lung of the dog whose liver was so extensively involved, a definite tubercle-like collection of monocyctic cells was seen. The failure to demonstrate bacilli of tuberculosis among the cells of these lesions leaves their true nature one of conjecture. Morphologically, however, they simulated rather

# THE PATHOLOGICAL CHANGES FOLLOWING EXPERIMENTAL EXPOSURE OF DOGS TO MYCOBACTERIUM TUBERCULOSIS OF AVIAN ORIGIN \*

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Although spontaneous tuberculous infections due to the bacillus of avian tuberculosis have been observed in many of the common mammals, including swine, horses, cattle and sheep, I have been unable to find in the literature reviewed a single instance in which the dog was affected. Since there are many opportunities for the average farm dog to become infected spontaneously with the avian form of the disease, his apparent failure to do so must be due to rather definite factors, but of an intangible nature.

In a series of experiments previously reported <sup>1</sup> it was shown that although the dog was extremely resistant to bacilli of avian tuberculosis by ordinary means of exposure, organisms injected directly into the cerebral substance initiated a definite tuberculous infection which was usually rapidly fatal. It was also noted that when large numbers of the organisms were introduced directly into the blood stream, tuberculous lesions of a mild type occasionally occurred. In my previous report I did not give a detailed account of the pathological changes observed, and therefore, these will be considered here.

The infective material used in these experiments consisted either of emulsions prepared from the liver and spleen of chickens which had died as a consequence of tuberculous infection, or of bacterial suspensions of bacilli which had been isolated in our laboratory by the method of Corper and Uyei, <sup>2, 3</sup> from spontaneous cases of avian tuberculosis. The experiments which were productive of definite lesions of a tuberculous character were those in which the organisms were injected intravenously or intracerebrally.

The jugular vein of ten adult dogs was injected with bacilli of avian tuberculosis. Only one of the animals died spontaneously; the others were killed for autopsy over a period of a hundred days to a

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excitant common to all. The lesions were distributed for the most part under the pia mater of the surface of the brain and in the sulci. In a few instances they were also observed as discrete foci in the substance of the cerebrum.

Anatomically the lesions of the pia mater seemed to have had their inception in the region immediately adjacent to the blood vessels. The lesions expanded diffusely in a perivascular manner, and in not a few instances perivascular collections of monocyctic cells were present in the depth of the cerebral tissue. All the blood vessels were highly congested, but parenchymatous retrograde changes of the cerebrum or cerebellum other than those in immediate contact with the specific cellular reactions were not apparent.

Examined minutely, the respective cellular reactions were of a diffuse character with no demonstrable tendency toward the formation of tubercles (Fig. 2). Most of the cells were of the usual monocyctic character, disposed in compact formation, and the only discernible stroma was the occasional remnant of the preëxistent connective tissue. Occasionally mitotic division of the monocyctic cells was seen. A few small lymphocytic cells were distributed promiscuously among the other elements, but polymorphonuclear leukocytes were absent.

In the sections appropriately stained, many acid-fast bacteria morphologically identical with *Mycobacterium tuberculosis* were present among the cells of practically all of the lesions studied. Many of these appeared singly with no significant relation to the cells constituting the lesions, whereas many others appeared in clumps which often occupied the cytoplasm of a monocyctic cell. Many of the phagocytosed bacteria were smaller than normal and the remains of many were represented by coccoid forms. For the most part, acid-fast bacteria were rather numerous throughout the tissues of the brain.

The Langhans' type of giant cell was not observed, although a careful search for it was made. The central portion of an occasional lesion was undergoing caseation necrosis although this type of retrogression was by no means commonly present. Calcification was not observed.

A study was made of the spinal cord of one of the dogs. The animal had succumbed twenty-four days subsequent to the intracerebral injection of bacilli of avian tuberculosis and sections were

closely lesions induced in the lung of another dog as a consequence of an injection of bacilli of tuberculosis of human origin.

By far the most significant lesions were observed in the tissues of the dogs that were exposed to bacilli of avian tuberculosis by the intracerebral method of inoculation. In this group there were six animals, each of which received intracerebrally 1 cc. of a heavy saline suspension of *Mycobacterium tuberculosis* of proved avian type. Three different strains of the organism were used and two dogs were injected with each strain. All the dogs given intracerebral injections died seventeen to twenty-nine days after the inoculation, and definite tuberculous lesions were readily demonstrated in the tissues of each dog.

In the brains of each of the dogs which had been given intracerebral injections there was no evidence of pyogenic infection being a contributory factor in the death of the animal. The operative wounds had healed and the frequent and regular recording of the temperature of each of the animals failed to disclose a febrile state such as one might expect if an infection had been present due to the ordinary pyogenic microorganisms.

On the removal of the part of the cranium necessary for the removal of the brain, more or less severe meningitis was observed. The dura mater was commonly adherent to the cranium at the point of the perforation incidental to the injection and a major portion of the membrane was usually congested. In a few of the animals the dura mater was adherent to the pia mater, particularly over the cerebral hemisphere receiving the inoculation, and the latter membrane was in a state of congestion in all instances. Hydrocephalus was not observed; in fact, the amount of fluid present was, if anything, considerably less than normal. The presence of acid-fast bacilli was demonstrated in smears made from the pia mater of the brains of all the dogs, and in cases in which cultures were attempted the results were successful.

Blocks were obtained for histological study from several different portions of each brain. Sections were stained with hematoxylin and eosin and carbol fuchsin and hematoxylin, the latter for the purpose of demonstrating the presence of acid-fast bacteria.

Microscopically a vigorous monocytic proliferation was present which was so consistent throughout all of the material studied as to warrant the conclusion that the reaction had been induced by an

Neither giant cells nor polymorphonuclear leukocytes were present and only an occasional lymphocyte was seen. Necrotic changes in the lesions were not apparent. Acid-fast bacilli, although rather scarce, were capable of demonstration within the lesions. Cultures of *Mycobacterium tuberculosis* were obtained from several of the livers.

### COMMENT

The methods used in inducing experimental infection of the dog with bacilli of avian tuberculosis have made it possible to obtain information concerning the manner in which the tissues of the dog respond to an infection by this organism. From the morphological point of view it has been possible, therefore, to study several factors of interest in the pathological concept of tuberculous infections.

The distribution of the lesions suggests the significance of the susceptibility of organs in the pathogenesis of tuberculosis, and at the same time leaves one without an adequate explanation of this little understood phenomenon. Although it was possible to establish a tuberculous involvement of the liver in dogs that were given injections either intracerebrally or intravenously with *Mycobacterium tuberculosis* of avian origin, the lungs seemed to escape the disease. There can be no reasonable doubt about the lungs having been exposed to the infective material by both routes of inoculation, particularly by the intravenous route in which the exposure was massive. Apparently, however, the residence of the microorganisms in this organ was not sufficiently prolonged to incite a cellular reaction demonstrable at the time of autopsy. The spleen likewise exhibited marked resistance to the infection.

Corper and Lurie<sup>4</sup> observed marked variability in the susceptibility of the various organs of the dog to human and bovine bacilli of tuberculosis, which they attributed to differences in the ability of the various phagocytic cells to destroy the offending organisms. They considered the extensive involvement of the liver as being due to the limited capacity of the Kupffer cells to destroy the bacteria, and the remarkable resistance of the spleen to the infection could be accounted for, they thought, by the ability of the "histiocytic elements of the spleen to effectually destroy the tubercle bacilli." The failure of the infective material in my experiments to establish definite lesions in the lungs may have been due to similar

obtained from the cervical, thoracic and sacral portions of the spinal cord. Although demonstrable involvement was not observed in the tissues from the sacral and thoracic portions examined, there were unquestionable lesions in tissues obtained from the cervical portion. The lesions which involved the pia mater extended entirely around the cord in a diffuse manner, but in no instance was the substance of the spinal cord violated. Solitary tubercles were not apparent (Fig. 3). The reactive elements constituting the lesions were similar to those concerned in the lesions of the brain except that the monocyctic cells were less compactly arranged and between the cells there seemed to be a little fluid. Polymorphonuclear leukocytes were missing, although small lymphocytes were fairly numerous. As in the lesions of the brain, giant cells were not seen nor were there any of the usual criteria of a reparative process. Acid-fast bacilli, although readily demonstrable, were not numerous. As far as could be determined, the dura mater was not implicated in the infective process.

Histological examination was made of the lungs, kidneys, pancreas, thyroid and suprarenal glands of the six dogs that had been given intracerebral injections, but tuberculous lesions were not found. In the spleen of one dog a few acid-fast bacilli morphologically identical with *Mycobacterium tuberculosis* were observed, but only a slight monocyctic reaction was observed.

The livers of all dogs in the series that were given intracerebral injections were found to possess definite tuberculous lesions. These consisted of multiple, discrete collections of monocyctic cells in the substance of the various lobules of the liver (Fig. 4). The respective foci seemed to have had their inception in the walls of the sinusoids and the lesions varied in number in the respective animals from numerous and extensive foci to an occasional collection of monocyctic cells. The majority of the lesions were vigorously proliferating and an occasional monocyctic cell in mitosis could be seen. The lesions appeared to be enlarging expansively at the expense of the adjacent hepatic cells which were gradually being replaced. The remaining cells of the parenchyma, except those in immediate contact with the lesions which were atrophic, were not visibly altered although all of the blood channels were congested. In none of the involved livers was there the slightest evidence that the lesions had induced any inhibitory influence whatever on that part of the liver.

typical tubercles, and necrosis was infrequently observed. Total absence of giant cells of the usual Langhans' type was noted. Even in the occasional lesions of the brain which were undergoing caseation necrosis, giant cells were not observed. I do not believe that the age of the lesions is of first importance in the development of giant cells in tuberculous lesions since I have observed these structures in tubercles in a rabbit's liver eleven days after the injection of bacilli of avian tuberculosis. Even in spontaneous tuberculosis of the dog due to organisms of human origin, giant cells do not seem to be a part of the morphological reaction.<sup>5</sup> These observations suggest that the Langhans' type of giant cell is not a part of the cellular response in tuberculous infections in the dog.

Although the dog unquestionably possesses a stubborn resistance toward *Mycobacterium tuberculosis* of avian origin when exposed by ordinary means, the morphological data in this study indicate that once the infection becomes established the respective monocytic cells of the lesions exercise considerable inherent ability to withstand retrograde changes successfully. This was evidenced by the minimal amount of necrosis observed and the absence of calcification even in lesions which are definitely atrophic.

## SUMMARY AND CONCLUSIONS

1. A detailed histopathological study was made of the tissues obtained from dogs which had been infected with bacilli of avian tuberculosis. Infection was accomplished by injecting the organisms directly into the brain in six animals, and an occasional infection was obtained by the introduction of large numbers of the organisms intravenously in ten animals.

2. In the dogs in which the infection was established by way of the brain, definite tuberculous lesions were found in the brain and liver in every instance. In only one animal was the spleen involved, and the lungs escaped demonstrable infection in each case. In the few animals in which the disease resulted subsequent to the intravenous route of inoculation, the lesions which were for the most part non-progressive were sharply limited to the liver. The information at hand seems inadequate to explain satisfactorily the consistent and extensive infection of the liver in the intracerebrally inoculated animals, in contradistinction to the infrequency of lesions in the

factors as those advanced by Corper and Lurie for the spleen. The failure of the disease consistently to promote extensive lesions in the liver of all dogs given intravenous injections, and the occurrence of multiple lesions of a progressive character in the livers of all dogs exposed by intracerebral inoculation cannot, however, be explained on the basis of the defense capacity possessed by the Kupffer cells. In fact the number of organisms injected intravenously was infinitely greater in every instance than those injected by way of the brain, and yet with one exception the lesions in the liver following the intravenous injections were quiescent and atrophic. Since the intravenous injections were given by way of the jugular vein, the possibility of the phagocytic cells of the lung engulfing and incapacitating the majority of the organisms before they could reach the liver is perhaps worthy of mention, but devoid of proof. On the other hand, in the intracerebral injections, organisms which were primarily established in the brain eventually gave rise to tuberculous foci in the liver and if they were transported from the brain to the liver by way of the blood stream one might reasonably expect the lungs to react in a manner comparable to that suggested in the animals that were given intravenous injections. That the lungs of all animals were free of definite demonstrable tuberculous lesions at autopsy rather forcibly suggests the possession in these organs of a most formidable defensive mechanism against the bacillus of avian tuberculosis, which is inadequate in the case of the human or bovine form of *Mycobacterium tuberculosis*. The exact mechanism of this resistance is worthy of further study.

Although cellular units identical with the typical miliary tubercle were not observed in the study of the various tissues of the dog in which there were lesions, this difference may have been due, at least in part, to the relatively immature age of the respective lesions in the majority of the animals. It should be noted, however, that there was very little structural difference between the lesions in the liver of the dog which died eighteen days after intracerebral exposure, and the lesions in the liver of the dog which was killed for autopsy a hundred days after an intravenous injection of the infective organisms. The lesions in both instances were essentially nothing more than compact, spherical or ovoid accumulations of monocyctic cells. The cells failed to exhibit any definite tendency to assume an elongated form such as is commonly observed in the "epithelioid" cells of many

## DESCRIPTION OF PLATES

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### PLATE 30

- FIG. 1. Accumulation of monocytic cells, many of which are definitely atrophic, in the liver of a dog killed for autopsy two hundred twenty-six days after the intravenous injection of bacilli of avian tuberculosis. Absence of encapsulating elements may be noted.  $\times 660$ .
- FIG. 2. Tuberculous lesion in the sulcus of the brain of a dog which died seventeen days after the intracerebral injection of bacilli of avian tuberculosis. The diffuse character of the reaction with no tendency toward the formation of tubercles may be noted. A few lymphocytes are present. Acid-fast bacilli were observed among the cells of the lesion.  $\times 100$ .

liver of the dogs inoculated intravenously. Likewise the failure of the disease to become manifest in the lungs is difficult of acceptable explanation and emphasizes the significance of the susceptibility of organs in the pathogenesis of tuberculosis.

3. The lesions induced were essentially circumscribed or diffuse accumulations of monocytic cells. As a rule, most of the lesions were progressive, and necrosis was not common. The apparent absence of giant cells would indicate that these structures are not a part of the histological response of the dog toward tuberculous infections.

4. Although the dog's brain offers a vulnerable portal of entry for the subsequent development of a tuberculous process by bacilli of avian tuberculosis, attempts to establish an infection with this organism by other means bring out the fact that the dog possesses an extremely formidable constitutional resistance to this form of the disease.

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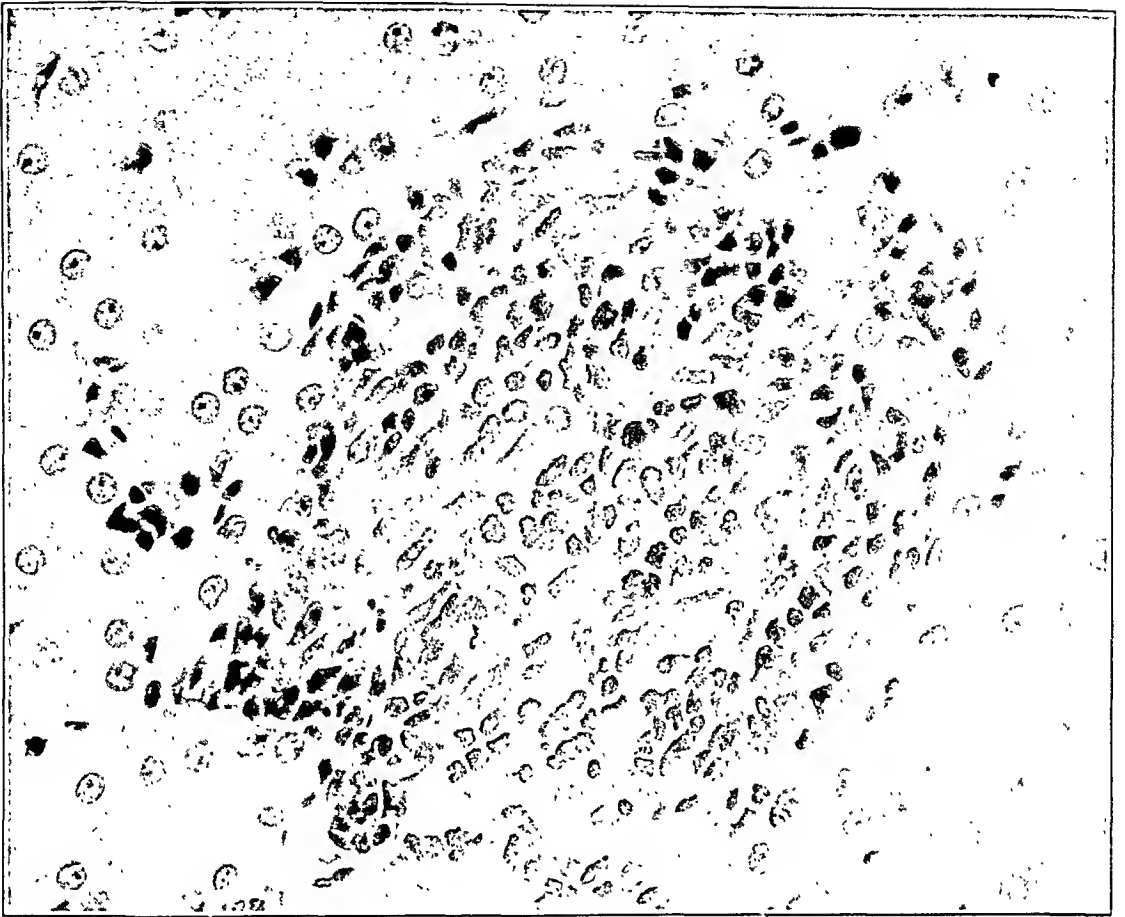
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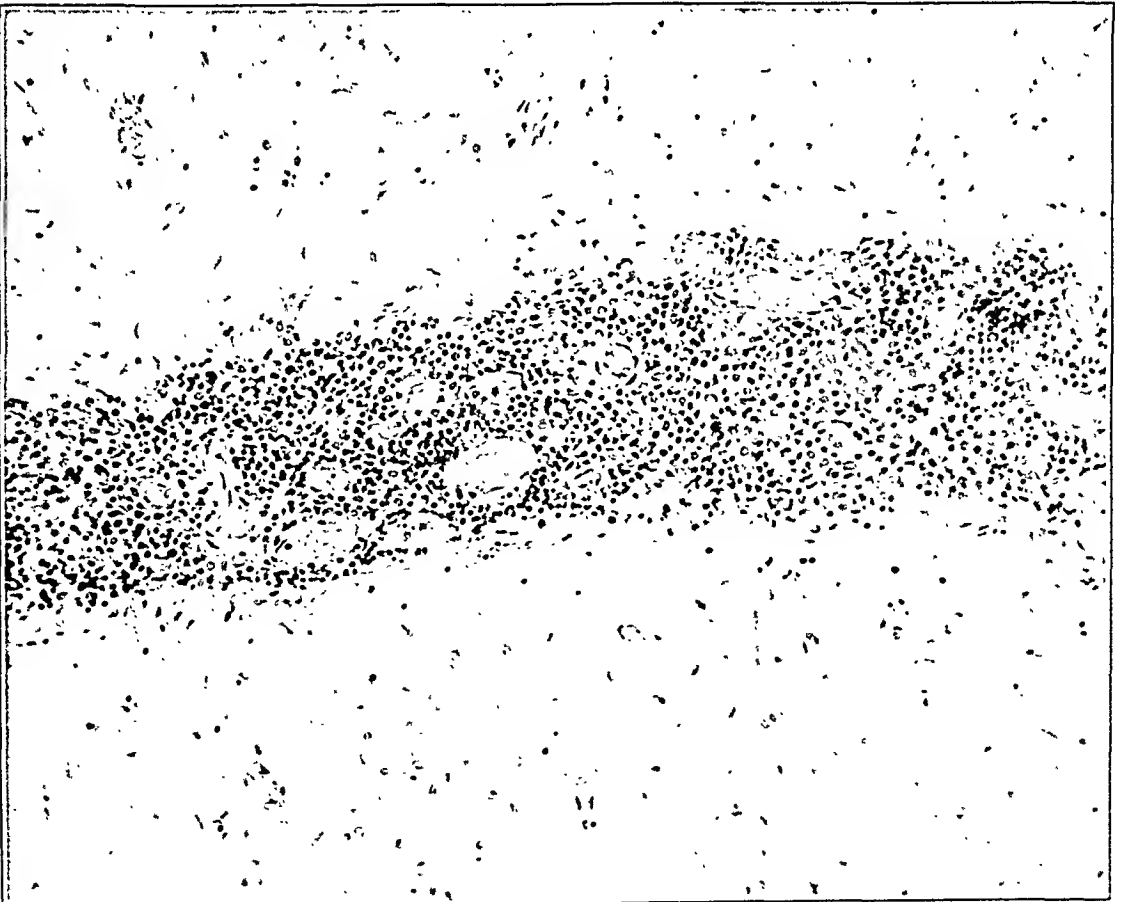
PLATE 31

FIG. 3. Tuberculous meningitis of the middle cervical portion of the spinal cord of a dog which died twenty-four days after the intracerebral injection of bacilli of avian tuberculosis. The cellular reaction was limited to the pia mater. Acid-fast organisms were demonstrated in the lesion.  $\times 200$ .

FIG. 4. Multiple tuberculous foci in the liver of a dog which died eighteen days after receiving an intracerebral injection of *Mycobacterium tuberculosis* of avian origin. The lesions were progressive and were promiscuously distributed throughout the organ.  $\times 220$ .



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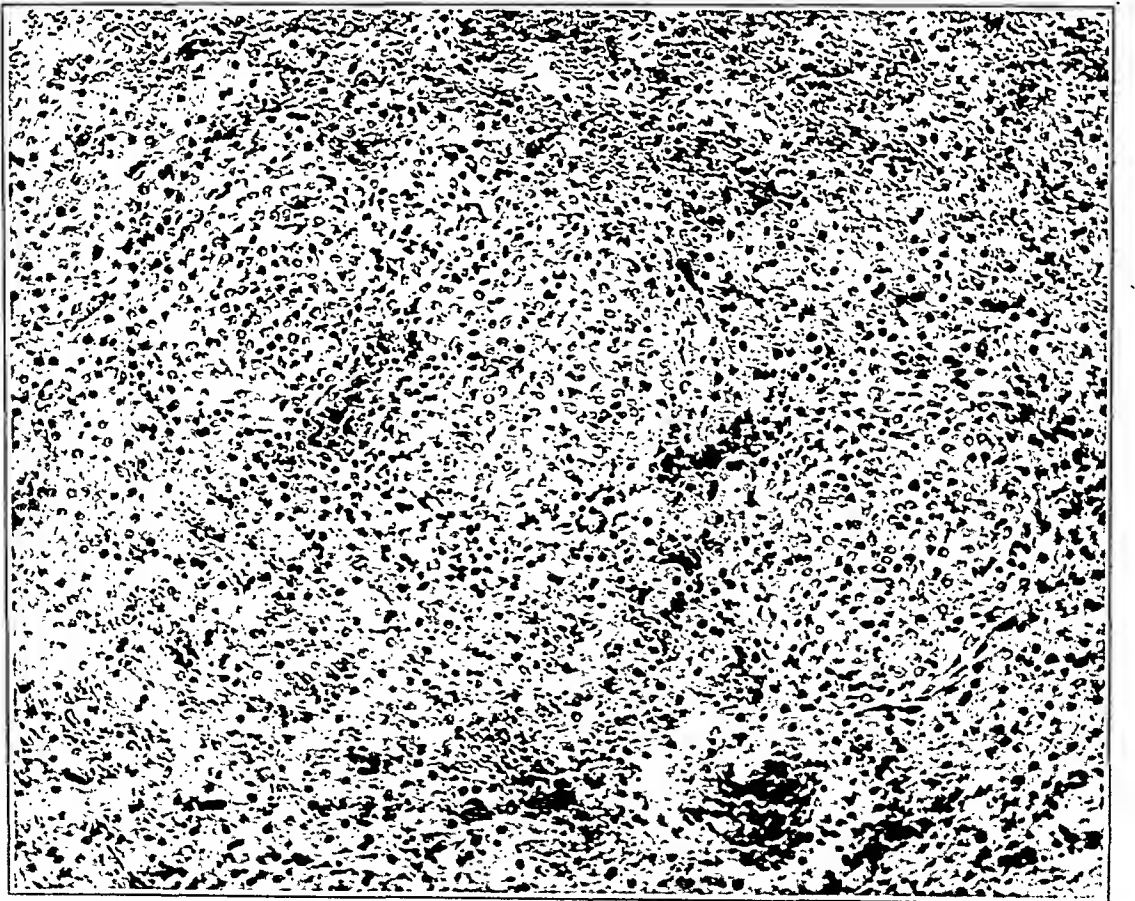


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not remarkable in gross. The endocardium was smooth and glistening. With the exception of the tricuspid, the valves were normal as were also the coronaries. Foramen ovale and ductus arteriosus were closed. The tricuspid valve (Fig. 1) presented on its upper surface at the margin, a small, pedunculated growth composed of many fibrous, yet delicate, lace-like villous processes, growing about the pedicle in a tree-like manner.

Microscopically, as can be seen in Fig. 2, this growth consists of multiple branching papillae which contain no blood vessels. Every branch or stalk of tissue is composed of four different structures. In the center is a single or compound core of closely compacted wavy bundles of delicate collagen fibrils. Only a few fibroblasts are present among them. These central cores are surrounded by a thin layer of loose collagenous material containing relatively more fibroblasts. Next comes a homogeneous layer of varying thickness in which a small number of cells often with branching cytoplasmic processes are present. In places the two outer layers are more or less intimately fused together. On the outside, covering every stalk, is a layer of endothelial cells, some of which are multinucleated and contain as many as eight nuclei.

Weigert's elastic tissue stain shows fairly numerous fibrils in places, both in the central core and more abundantly in the loose connective tissue layer covering it. None can be found in the homogeneous layer. Mallory's phosphotungstic acid hematoxylin stain shows fibroglia fibrils to be present fairly numerous in the loose connective tissue layer, but also to some extent in the central cores and in the homogeneous layer. A silver stain demonstrates reticulum fibrils in the two outer layers.

There is no evidence anywhere of old fibrin undergoing organization.

In a few places a little fresh fibrin and small numbers of red blood corpuscles occur in clefts in the two outer layers. A few leucocytes are also sometimes present. These elements would seem to have been included in these layers during the process of growth.

Underneath the endothelium lining the valve is a homogeneous layer like that covering the papillary stalks of the tumor. It abuts directly on a rather dense layer of connective tissue. In it in places are delicate fibroglia fibrils.

In conclusion it may be helpful as well as interesting to summarize

## PRIMARY NEOPLASM OF HEART VALVE \*

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Primary neoplasms of the heart valves, although usually of no clinical importance, are of interest because of their great rarity. Less than a score have been reported to date.

We may note here that myxomas have been reported by Debove,<sup>1</sup> Djewitzky,<sup>2</sup> Guth,<sup>3</sup> Leonhardt,<sup>4</sup> and Ribbert.<sup>5</sup> Fibromas have been reported by Curtis,<sup>6</sup> Forel,<sup>7</sup> Reitmann<sup>8</sup> and Ribbert, while Forel claims to have found the only endothelioma to date. These authors have carefully recorded all of their findings in these cases and each insists that his tumor is a true neoplastic growth. These tumors are not to be confused with the hemorrhagic nodules occasionally noted on the heart valves of infants. Koechlin<sup>9</sup> was the first to note and question the origin and classification of these "Blutknötchen." Since that time many authors have reported this type of growth, both in infants and adults. The papers of Wendel<sup>10</sup> and Wegelin<sup>11</sup> form a comprehensive survey of this particular entity.

Lambl<sup>12</sup> in 1856 wrote of papillary excrescences on the aortic valve; these were perhaps of the true tumor group, although some authors seem to feel that they represented only sclerosed hemorrhagic nodules.

It seems from a scrutiny of the many detailed reports that some of the alleged neoplasms perhaps more rightfully were persistent blood nodules, while it is equally possible that a few of the latter, particularly Lambl's group, were true neoplasms.

The tumor I wish to report was discovered in the routine course of an autopsy performed on a woman, 35 years of age, who had a fatal secondary hemorrhage following hysterectomy. No essential pathology, other than the hemorrhage, was present.

The heart weighed 275 gm., and measured 13 by 9 by 4.5 cm. The epicardium was smooth, glistening and covered a small amount of subepicardial fat. The myocardium was firm, pale red-brown, and

\* Received for publication January 27, 1931.

## DESCRIPTION OF PLATE

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### PLATE 32

- FIG. 1. Tricuspid valve showing on its upper surface a delicate branching vil-  
lous tumor.
- FIG. 2. Branching stalks of tumor tissue composed of single and compound  
cores of dense collagen surrounded by loose connective tissue and a homo-  
geneous layer covered with endothelial cells.  $\times 40$ .

the few statistics that can be compiled at this time. As far as we can learn there are now on record thirteen cases of primary neoplasms of the heart valves, excluding the blood nodule group and Lambl's papillary excrescences. They are all small, benign and clinically unimportant. The sex was reported in eight cases; seven were females. The youngest was 22 years of age, the oldest 83. Incidence of occurrence on the valves was as follows: tricuspid valve 6, mitral valve 3, pulmonic valve 2, aortic valve 2. Seven were avascular in nature. Tumor types encountered were myxoma 6, fibroma 6, endothelioma 1.

### SUMMARY AND CONCLUSIONS

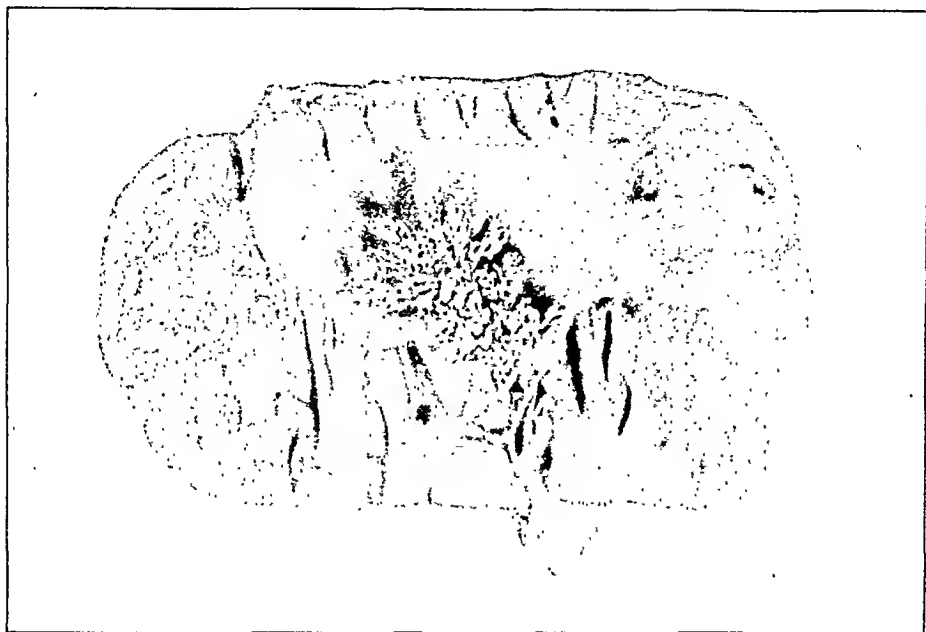
A papillary branching fibroma of the tricuspid valve is described. The tumor, like the valve, contains no blood vessels. Every papillary stalk of the tumor is composed of a single or compound core of dense collagen fibrils surrounded by loose connective tissue, outside of which is a layer of homogeneous material covered by endothelium. The tumor seems to show a gradual growth and transformation of fibroblasts, starting in the homogeneous layer and ending in dense fibrous tissue. A similar homogeneous layer occurs beneath the endothelium lining the valve.

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amination failed to elucidate any clearly defined focus of consolidation, although there was some dullness in the left upper chest. The fever ranged from 101 to 104 F. The patient failed steadily and died, rather suddenly, without marked change in pulse or respiration, October 27, 1930, at 11.30 A.M. Autopsy was performed two hours later.

### POSTMORTEM EXAMINATION

*Body:* Is that of a well developed, somewhat emaciated, young, adult white male. There is a well healed scar 10 cm. long along posterior crest of left ileum. Whole left side of pelvis from sacrum posteriorly to symphysis pubis anteriorly is markedly larger than right. A large subcutaneous, thick, slightly fluctuant mass can be palpated beneath skin. There are several superficial ulcerations in skin over buttocks. Moderate pitting edema of left ankle and legs. Left thigh larger than right. Rigor mortis absent. No icterus or distention.

*Head:* Moderate growth of short black hair. Pupils equal, regular, 6 mm. in diameter. Ears and nose negative. Teeth in fair condition.

*Primary Incision:* Y type. Panniculus adiposus 1 cm. thick.

*Peritoneal Cavity:* Surfaces smooth and glistening. No adhesions. Sigmoid is pushed anteriorly by a large tumor mass filling left iliac fossa.

Appendix present, retrocecal and normal. Diaphragm fourth rib right, fourth interspace left.

*Pleural Cavities:* Right negative. No adhesions or free fluid. Left cavity contains a few easily broken adhesions between upper lobe and chest wall. No free fluid.

*Pericardial Cavity:* Surfaces smooth and negative. Contains 30 cc. of clear yellow fluid.

*Heart:* Weight 290 gm. Epicardium negative. Opened *in situ*. Myocardium firm, dark reddish brown. Valves and endocardium negative except that pulmonary valve is composed of four cusps. Foramen ovale closed. Coronaries negative. Pulmonary artery clear but palpation detects a firm obstruction in left and right branches. Measurements: Tricuspid valve 12.5 cm., pulmonary valve 6.5 cm., mitral valve 8 cm., aortic valve 6.5 cm., left ventricle 1 cm., right ventricle 0.2 cm.

*Lungs:* Left lung: surface shows a roughened focus on upper lobe, adherent to parietal pleura. Beneath this is a rounded, markedly

## CHONDROSARCOMA WITH INTRAVASCULAR GROWTH AND TUMOR EMBOLI TO LUNGS \*

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While the bizarre intravascular growths of chondrosarcoma have long been known,<sup>1, 2</sup> a case recently observed in this laboratory has sufficiently unusual features to warrant reporting it. There is rather marked similarity between this present case and one reported by Ernst,<sup>3</sup> an enchondroma originating in the twelfth thoracic vertebra, which had invaded the renal and suprarenal veins, the vena cava and the left ovarian vein. There were masses of the tumor in both pulmonary arteries, in the right a bifurcated mass of tumor tissue, whereas the one lodged in the left pulmonary artery had extended into the branches of the artery so that it had a hand-like appearance. There were no true metastases. In Ernst's article references to the earlier cases are given.

### REPORT OF CASE

*Clinical History:* W. Y., a white male, 32 years of age, was admitted to the New England Deaconess Hospital May 20, 1930, under the care of Dr. Dwight L. Sisco, complaining of pain and soreness in the lower back. Three weeks before he had begun to have pain in the left gluteal region, which he thought was due to muscular lameness because of unusual exercise. On physical examination a walnut-sized nodule was palpable just above the left sacro-iliac joint. The examination was otherwise negative. An abnormal rarefaction of bone about the left sacro-iliac joint was found on X-ray examination. The lungs were clear. May 24, 1930, under gas anesthesia, the tumor was explored by Dr. William A. Rogers and found to be a chondrosarcoma. July 5, 1930 the patient was transferred to the Palmer Memorial Hospital, under the care of Dr. E. L. Daland. A course of deep X-ray therapy was given. The patient steadily lost strength and had considerable pain. October 8, 1930, at 7 P.M. the patient had a sudden attack of pain in the precordial region, which was extremely intense and not controlled by morphia. The respirations increased slightly. An X-ray of the chest on this date showed diffuse clouding of the lung in the third left interspace, which suggested a focus of metastasis. October 11, 1930, physical ex-

\* Received for publication January 21, 1931.

thickened, measuring 1 to 1.3 cm. in diameter and composed of whitish gray, firm, fibrous material.

*Prostate:* Negative.

*External Genitalia:* Negative.

*Lymph Nodes:* Along course of abdominal aorta are several enlarged nodes 1.5 cm. long, which on section are pale gray, firm and somewhat inelastic.

*Veins:* Inferior vena cava just above junction of iliac vein is blocked by a whitish gray, firm, cartilaginous mass which continues into smaller and larger tributaries of inferior vena cava, especially on left side. This growth is slightly adherent to vessel walls. On left side it extends as far as saphenous junction with femoral vein and into hypogastric vein. The latter is surrounded by tumor tissue. Right common iliac vein contains similar material as well as a slight amount of ante mortem thrombus.

*Aorta:* A few foci of atheromatous change.

*Tumor:* Beneath iliopsoas muscle in left iliac fossa is a large oval tumor measuring 17 cm. long and 10 cm. in diameter, extending from level of fifth lumbar vertebra above, to beneath the inguinal ligament below. This mass is firmly fixed and slightly fluctuant. Section through mass reveals a coarsely honeycombed, whitish gray structure with cavities filled with a thick, mucoid, yellowish brown fluid. Center of mass markedly cystic. Exact posterior limits of this mass cannot be determined but it involves sacrum, left side of bony pelvis, but not lumbar vertebrae.

*Anatomical Diagnoses:* Chondrosarcoma of left sacro-iliac synchondrosis with extension into common iliac veins, left femoral and left hypogastric veins and inferior vena cava; metastases to lungs; tumor embolus, left pulmonary artery; pyonephrosis, bilateral; pyelonephritis, bilateral; multiple abscesses of kidneys and left lung; pyo-ureter, bilateral; chronic cystitis; chronic passive congestion of liver; anomaly of pulmonary valve; edema of left leg; decubiti.

#### MICROSCOPIC EXAMINATION

*Heart:* Slight increase in connective tissue between muscle bundles.

*Lung:* Vessels are congested. In most areas the alveolar walls are somewhat broken near pleural surface. One section shows a small

resilient focus. On section this is found to be filled with air. Cavity entered measures 5 cm. in diameter and inner wall is roughened and covered with greenish, foul, purulent exudate. Wall of cavity 3 to 4 mm. thick. Three similar smaller cavities found in surrounding lung tissue. Left lower lobe negative. Left pulmonary artery completely occluded by a whitish gray, firm, cartilaginous mass which extends and ramifies to smaller branches of pulmonary artery in left and lower lobes. This is somewhat adherent to walls of vessels. Some of smaller branches of right pulmonary artery contain bits of whitish, cartilaginous material similar to that found in left pulmonary artery.

Right lung: contains several firm nodules up to 2 cm. in diameter, which on section show a firm, pale gray, glistening, inelastic structure. These are close beside or involving branches of the pulmonary artery.

*Spleen:* Weight 210 gm. Capsule smooth and dark reddish. Cut surface rather firm and dark reddish with distinct markings. Almost no pulp is scraped away.

*Pancreas:* Weight 60 gm. Normal in size and consistency. On section pale gray and elastic with normal lobulations.

*Gastro-Intestinal Tract:* Negative.

*Liver:* Weight 2080 gm. Surface reddish brown and smooth. On section, edges evert slightly. Cut surface chocolate brown with distinct lobulations and slightly congested central areas. Gall-bladder negative. Bile ducts patent. No stones present.

*Adrenals:* Negative.

*Kidneys:* Weight, right 290 gm., left 290 gm. Capsule strips easily from an irregular, dark reddish gray surface mottled with numerous yellowish foci measuring 2 to 4 mm. in diameter. Section of right kidney reveals a slightly dilated pelvis filled with thick, creamy, yellowish pus. Pelvic mucosa markedly injected. Cortex and medulla contain numerous discrete yellowish foci. There is poor differentiation between cortex and medulla.

Left kidney: on section similar to right, but contains fewer abscesses.

*Ureters:* Both are dilated and measure about 0.5 cm. in diameter. Filled with thick, creamy, yellowish, purulent material.

*Bladder:* Rather firmly contracted. Section reveals a dark reddish, markedly injected and roughened mucosa. Bladder wall

scattered tumor giant cells. In a few foci a considerable amount of hemosiderin is present. One point in section shows a large vein almost completely filled by mass of tumor tissue, which is not adherent to the vessel wall except at one or two points. The intravascular portion of the growth does not vary in character from the extravascular. In several places apparently intact nerves are seen passing through the tumor. The tumor tissue itself is relatively avascular, except at the periphery.

*Lymph Node:* Section through aortic lymph node shows an increase in connective tissue in lymphoid follicles. No tumor cells are observed. Lumbar nodes show quite marked increase in connective tissue.

*Aorta:* Section examined shows slight amount of lipoid material in subintimal layer.

## DISCUSSION

The extent of intravascular growth of this tumor is extraordinary. Apparently the tumor gained access to the venous system through the branches of the left hypogastric vein and from there readily grew into the common iliac vein, extending on the one hand up into the vena cava and on the other hand down the left external iliac vein into the femoral vein. The intravascular tumor mass was of interest in that it lay, for the large part, free within the lumen of the vessel, being only slightly adherent. The cells of the tumor within the veins were in the main well differentiated and produced a very large amount of cartilage matrix.

Apparently on October 8, so far as can be judged from the clinical history, a considerable portion of the tumor mass broke away from the mass within the vena cava and was swept by the blood stream through the right heart into the left pulmonary artery, where it lodged. Apparently not disconcerted by this sudden change of locus, it continued its growth in various branches of the pulmonary artery until death of the host. In curious contrast to the minute fragments of the tumor which gave rise to typical pulmonary metastases with destruction of the lung parenchyma, the outgrowths of tumor tissue from this large embolic mass did not invade the lung tissue.

necrotic area. Considerable fibrin, red blood cells and polymorphonuclear leucocytes in surrounding area. Pleural surface thickened by old granulation tissue. In some foci there is considerable anthracosis. Moderately large focus of lung tissue replaced by mass of well differentiated cartilage cells with considerable amount of matrix, some of which has undergone cystic degeneration. Considerable portion of mass is apparently within a blood vessel and some fibrin and red blood cells are present near the periphery. The tumor cells near the periphery are relatively undifferentiated, being of a plump spindle shape with only slight amount of intercellular substance. Mitoses are not infrequently seen. The surrounding lung tissue is compressed.

*Spleen:* Shows some congestion of sinusoids. Polymorphonuclear leucocytes are quite numerous in sinusoids.

*Pancreas:* Islet cells and acinar cells stain clearly. Lobules appear normal.

*Liver:* Sinusoids dilated and liver cords show some compression. In some areas liver cell nuclei are pyknotic. Cytoplasm is swollen and has a granular appearance.

*Kidney:* Numerous foci of polymorphonuclear leucocytes with necrotic centers are found. Glomerular tufts are congested in places and infiltrated with occasional polymorphonuclear leucocytes. Bowman's capsule distended and in many places red blood cells and mononuclear leucocytes are found in intracapsular space. Tubule cells show granular degeneration. Many nuclei are pyknotic. Tubules in many areas are filled with red blood cells. In a few areas near cortex there is a diffuse infiltration of interstitial tissue with polymorphonuclear leucocytes, mononuclear leucocytes and lymphocytes.

*Adrenal:* There is some necrosis in zona fasciculata of adrenal. Considerable congestion of vessels.

*Bladder:* Muscle wall appears normal. Mucosa is thickened and submucosa is infiltrated with lymphocytes and polymorphonuclear leucocytes in places. No evidence of tumor growth in this section.

*Tumor:* Bulk of tumor consists of somewhat atypical cartilage cells varying markedly in size, embedded in a considerable amount of matrix. Portions of matrix show extensive foci of cystic degeneration. Toward periphery numerous plump spindle-shaped, rounded cells varying markedly in size with fairly numerous mitoses and



## DESCRIPTION OF PLATE

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### PLATE 33

FIG. 1. Portion of main tumor mass above with tumor growing within internal iliac vein, shown continuous with tumor mass in left external iliac and femoral veins. Small projection at extreme left lay within the left saphenous vein. Portion to right lay within the common iliac vein and first 2 cm. of vena cava.

FIG. 2. Drawing, natural size, of tumor embolus from left pulmonary artery, showing extensive intravascular growth from embolic mass.

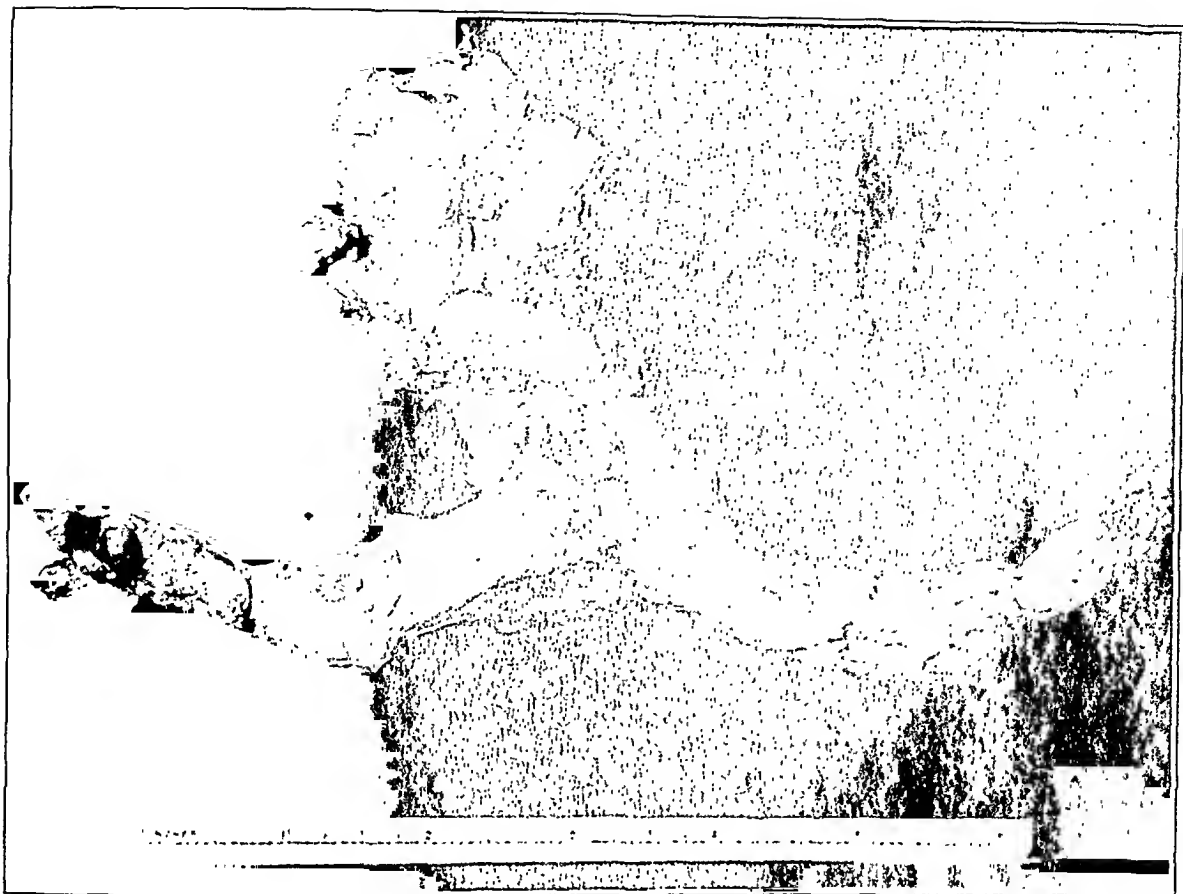
## SUMMARY

A case of chondrosarcoma with extensive intravascular growth and tumor emboli in the pulmonary circulation is described.

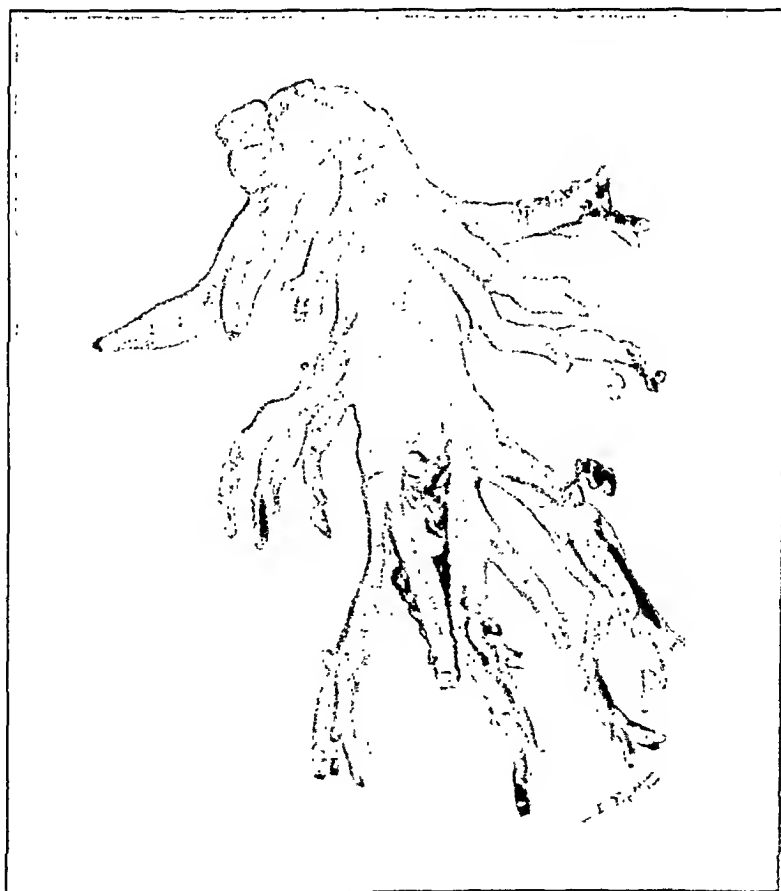
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in 1907, showed that crushing of the pancreas is associated with occlusion of the blood vessels, and with changes which resemble those of hemorrhagic necrosis. Mallory,<sup>7</sup> on the other hand, explained the occlusion of the pancreatic blood vessels in hemorrhagic pancreatitis by the action of the pancreatic enzyme on the walls of the vessels which he said produced thrombi.

Mann and Giordano<sup>8</sup> in a study entitled "The bile factor in pancreatitis," found the anatomical arrangement of the common bile duct and pancreatic duct to be such that the possibility of gallstones producing obstruction at the outlet of the papilla, to form a continuous channel from the common bile duct into the pancreatic duct, was small. They found that each duct opened separately into the duodenum in 31 per cent of cases. In 45 per cent the ducts united 0.2 cm. from the apex of the ampulla of Vater. In 20 per cent the ducts united 0.3 cm. to 10 mm. from the apex of the ampulla of Vater. In 4 per cent the pancreatic duct was absent or was reduced to a fibrous cord. They concluded that obstruction is possible in 20 per cent of cases providing the stone is larger than 3 mm. in diameter. If less than 3 mm. in diameter the possibility of obstruction being caused is slight, because the outlet of the papilla is usually 2 to 3 mm. in diameter and the stone might pass through without much difficulty.

Grant,<sup>9</sup> in 1928, reported twelve cases of acute pancreatic necrosis. Eleven of the twelve patients were operated on. Nine had stones in the gall-bladder. One had a single stone in the ampulla and one had a normal gall-bladder and stones were not found in the extrahepatic biliary tract. The reports of autopsy are indefinite as to the condition of the pancreas and of the outlet of the common bile duct and pancreatic duct. Hemorrhage in the pancreas was mentioned in only two of these cases.

Holzapfel,<sup>10</sup> in a more recent report, reached conclusions similar to those of Mann and Giordano, that hemorrhagic pancreatitis due to gallstones is possible in 20 per cent of persons because of the anatomical arrangement of the terminal portions of the common bile duct and pancreatic duct. In 80 per cent he found the common bile duct and the pancreatic duct each to have a separate opening into the intestine, either in the same or separate papillae. He expressed the same opinion as Opie that bile enters the pancreatic duct and activates the zymogens, thus producing hemorrhagic necrosis.

## HEMORRHAGIC PANCREATITIS \*

### REPORT OF TWO CASES IN WHICH GALLSTONES COULD NOT BE CONSIDERED ESSENTIAL ETIOLOGICAL FACTORS

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Review of the literature reveals that hemorrhagic pancreatitis was studied by Rokitsansky<sup>1</sup> in 1861, by Klebs<sup>2</sup> in 1869 and by Fitz<sup>3</sup> in 1889. They gave no explanation as to the etiology of the condition. They did, however, bring out the fact that hemorrhage in the pancreas, except when due to severe abdominal trauma, was usually associated with disease of the gall-bladder and bile passages. Many other observers during this period reported single cases in which hemorrhage had been observed in the pancreas, but their reports are too inadequate and inconclusive to be of much value.

Any suggestion as to the etiology of this condition, however, was not mentioned until 1901, when Opie<sup>4</sup> published his first account of a case, showing the relationship between pancreatic necrosis and impaction of a calculus in the ampullary portion of the common bile duct. Following this observation other cases were reported in which similar conditions were associated with acute hemorrhagic pancreatitis. Numerous experiments then followed in which it was attempted to reproduce this type of lesion in animals. The result of these experiments was the discovery that apparently the changes sought could be produced by injection of a variety of irritating substances, such as zinc chloride, artificial gastric juice, hydrochloric, nitric and chromic acids in varying strengths, and solutions of bacterial toxins, into the duct of Wirsung. Various oils also have been used, and all of these produced the same results. Various bland substances, however, such as blood, blood serum, agar-agar, paraffin, and emulsions of starch did not produce these changes.

Hlava,<sup>5</sup> in 1898, suggested that in human subjects hyperacid gastric juice may be forced by antiperistalsis of the intestine into the pancreatic duct, thus producing hemorrhagic pancreatitis. Levin,<sup>6</sup>

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base of the mesentery, to be studded with small, yellowish white, opaque spots of fat necrosis. There was hemorrhagic discoloration beneath the peritoneum around the pancreas. The gall-bladder contained 50 cc. of bile and there was slight cholesterosis of its mucosa. Gallstones were not present. The common bile duct appeared normal throughout. There was no evidence of stones, strictures or injury due to stones. In the middle of the pancreas were hemorrhagic regions. There was also more or less diffuse hemorrhage throughout the remainder of the gland. There were marked deposits of adipose connective tissue in the head and tail of the gland. These revealed typical, small, yellowish, opaque spots of fat necrosis. The pancreatic duct appeared to be normal throughout. Its outlet into the intestine was found to be separate from, and 5 mm. distal to, the outlet of the common bile duct.

Microscopic study of the pancreatic sections disclosed essentially the same changes as those which were present in Case 1.

#### COMMENT AND SUMMARY

In these two cases impaction of gallstones at the ampulla of Vater could not be considered an etiological factor in producing hemorrhagic disease of the pancreas. In Case 1, as shown in Fig. 2, the common bile duct and the pancreatic duct each had a separate opening on the tip of the papilla. In Case 2, the outlet of the pancreatic duct was 5 mm. distal to that of the common bile duct. There is no possibility in either case that the obstruction at the outlet produced a continuous channel from the common bile duct into the pancreatic duct.

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Judging from the literature the majority of authors seem to be of the opinion that a gallstone impacted in the ampulla of Vater converts the pancreatic and common bile ducts into a continuous channel and thereby diverts the flow of bile into the duct of Wirsung. The interaction of the bile with the pancreatic juice is believed to liberate an enzyme, probably trypsin, which is responsible for the lymphatic and vascular changes. In the cases reported here the anatomical arrangement of the pancreatic and common bile ducts at their terminations, does not give support to this theory.

### REPORT OF CASES

CASE 1. The history and examination in the case of a woman aged 57 years were suggestive of acute hemorrhagic pancreatitis.

At the postmortem examination the gall-bladder contained about 40 cc. of dark, greenish, viscid bile and more than 200 stones, varying in size from a diameter of 3 to 4 mm. to that of fine granules of sand. The wall was soft and slightly thickened, but grossly it did not show signs of active inflammation. The common bile duct contained several of these small stones, but was not dilated and did not show evidence of previous injury from stone (Fig. 1).

The pancreas weighed about 120 gm. and was increased in consistence throughout. On section two large hemorrhagic portions were found confined to the central portion of the gland. The pancreatic duct passed through these hemorrhagic regions. It was not dilated or obstructed and did not contain bile or other foreign substances. The common bile duct and the pancreatic duct each had a distinct and separate opening in the tip of the papilla (Fig. 2). There was no evidence that there had been obstruction at any time, for both ducts appeared to be normal at their outlets.

Microscopic examination of the pancreas revealed the usual appearance of hemorrhagic pancreatitis with destruction of pancreatic tissue. Numerous thrombi in various stages of organization were found in the veins.

CASE 2. A woman, aged 57 years, consulted the clinic because of a carcinoma of the left breast. Hemorrhagic pancreatitis was not suspected either before or after the operation for this condition.

The postmortem examination revealed the peritoneum in the region of the pancreas, below the transverse mesocolon and along the



## DESCRIPTION OF PLATE

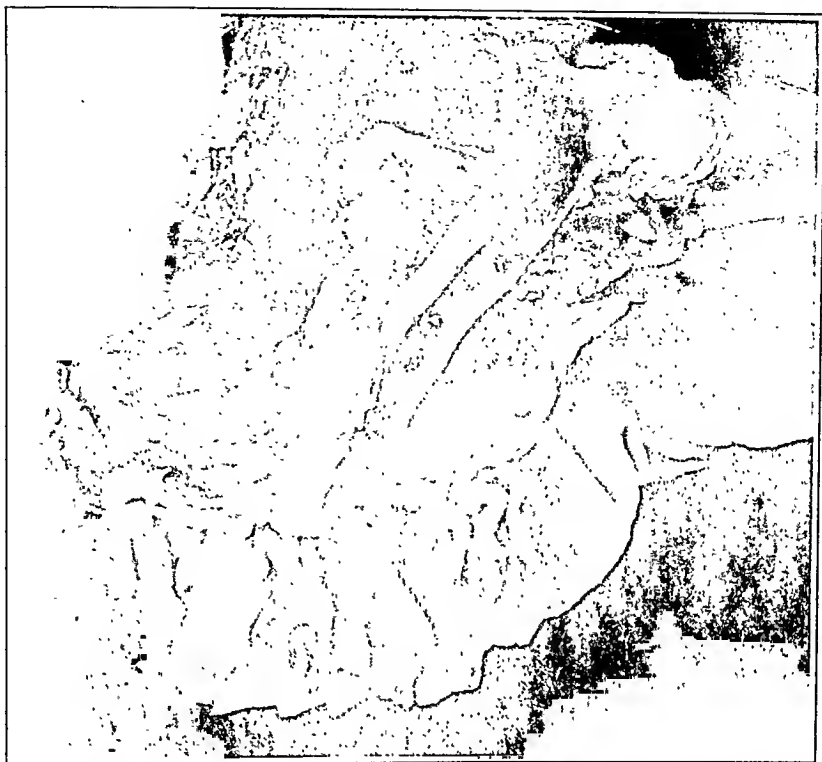
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### PLATE 34

- FIG. 1. Case 1. The common bile duct, containing many gallstones. This specimen is the same as that shown in Fig. 2.
- FIG. 2. Case 1. Probes in the common bile duct and pancreatic duct. The common bile duct and the pancreatic duct each opens separately into the outlet of the papilla.

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testines of swine and their relation to extensive mucosal destruction and regeneration following bacterial or parasitic invasions. Port<sup>7</sup> reviewed a series of multiple intestinal polypoid adenomas and concluded that the condition could be traced to a familial disposition and that it is not a disease developing later.

Hauser<sup>8</sup> divides the adenomatous proliferations into two groups. The first consists of those in which there is a degeneration of the epithelium, loss of physiological function and an absence of goblet cells. The widespread nature of this form suggests to him a contagious or living form as the causal factor. As an example of this he cites the extensive bile duct proliferations in rabbits due to coccidia, but because protozoan forms were not consistently found throughout the intestinal adenomatous areas he ruled them out. He believes irritation may favor further growth in this group, as occurs at the curvatures of the intestine in diffuse intestinal adenomatosis, but that irritation does not act as a primary factor. His second group includes newgrowths in which the epithelium resembles that of a normal mucous membrane, and in which mucin production is increased. These he considers as a simple gland hypertrophy with increased function secondary to a chronic inflammatory process.

Ruffer<sup>9</sup> describes tumors in the intestine due to oxyuris. Eggs were found in these growths but apparently the tissues were not studied histologically. Borst<sup>10</sup> attributes intestinal adenomatous polypi to unused germ cells which remained latent during development, or to those which were misplaced. In his opinion irritation can arouse adenomatous growths but only in tissues disposed to tumor growth. Milton<sup>11</sup> records polypous formations in connection with bilharziosis. Hart,<sup>12</sup> on the basis of a described case, holds it probable that intestinal polypi originate as a result of irritation or stimulation by a carcinomatous metastasis. Doering<sup>13</sup> states that in most cases the etiology is not understood. He confirms the observation of others that it is a disease of the young and middle-aged. Funkenstein, Janowski and Askanazy, cited by Doering,<sup>13</sup> found no evidence of a relationship between this process and protozoa.

Verse<sup>14</sup> distinguishes between a congenital anomaly and a congenital disposition of the epithelium to increased proliferation similar to that found in xeroderma pigmentosum. Preparations containing hyperplastic glands in which goblet cells are being displaced by undifferentiated epithelium are submitted as evidence of a probable

## INTESTINAL ADENOMA IN SWINE \*

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The ultimate result of epithelial regeneration following extensive destruction of mucosal tissue in infectious enteritis, and the degree of functional ability possessed by such regenerated epithelium seem of considerable importance in connection with the probability of recovery.

Kaufmann <sup>1</sup> notes that in severe cases of diphtheric enteritis associated with extensive destruction of tissue followed by the healing of ulcers, the remaining islands of mucosa may undergo glandular proliferation and produce intestinal polypi.

In spite of the greater opportunities for study, very little is known regarding the significance of adenomatous polypi in the intestines of domesticated animals. Petit and Germain <sup>2,3</sup> describe adenomatous growths in the stomach of the horse, due to the presence of *Ha-bronema megastoma* Rud. 1819 (syn. *Spiroptera megastoma*) and *Trichostrongylus axei*.

Neumann <sup>4</sup> attributes intestinal tumors from the size of a pinhead to a small walnut to sclerostomes. However, he makes no reference to the histological structure of these growths. Bergmann <sup>5</sup> noted in the rectum of a 7 month's old pig, pea- to walnut-sized growths composed of simple or branched gland ducts. He found necrophorous organisms in the growths and believed that they could not be ruled out as the primary cause. Henk <sup>6</sup> reported adenomas in the large intestine of the pig, followed by hemorrhages. Numerous clinical and autopsy reports by European army veterinarians record the presence of adenomatous growths in the intestines of the horse, but their etiology was not studied. With the exception of a few case reports not readily accessible, no mention is made of intestinal adenomas of the pig.

In view of the extensive studies on this subject in the human, a brief consideration of some of the outstanding work seems indicated in connection with a study of adenomatous newgrowths in the in-

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sized area of pneumonia and edema was found in the apex of each lung. The mucosa of the stomach was rough and thickened, suggesting a previous acute gastritis. The small intestine was devoid of food, anemic and its villi prominent. The wall of the ileum was slightly hypertrophied. The ileocecal valve was completely overgrown by a mass of polypi (Fig. 1). The wall of the cecum was greatly thickened, the mucosa in most places being obliterated by diffuse polypous growths. Many of these tumors were round or irregular, some being over one-half inch in diameter. The large colon presented a similar picture with the exception of the last 18 inches. Beyond this point appeared smaller growths, some suggesting proliferations of the glands in folds of the mucosa. Posterior to this region only scattered esophagostomum nodules were present. The small colon and rectum possessed a yellowish gray mucosa with an absence of gross tumors. The cecal and colic lymph nodes were greatly enlarged. The cut surfaces bulged and presented a yellow and gray mottled appearance. The splenic follicles were enlarged. Other organs showed no significant changes.

*Microscopic Examination:* The stomach showed a catarrhal gastritis of considerable standing, but an absence of gland proliferation. The small intestine presented a marked interstitial cellular infiltration with intact mucosa and an absence of new gland formations. The polypous growths involving the ileocecal valve, cecum and large colon consisted of adenomatous tissue. The new-formed glands were much larger than normal intestinal glands, the epithelial proliferation producing lateral branches or blind sacs (Figs. 2 and 3). Often the proliferating glands were forked and winding or they formed tree-like structures. Goblet cells were uniformly absent in the new-formed glands constituting the polypous growths. In their place were found undifferentiated, tall, columnar non-mucin-containing cells which were closely packed. Often the nuclei were stratified (Fig. 3).

In a number of preparations examined, single goblet cells were rarely noted in the midst of extensive adenomatous tissue proliferations composed of undifferentiated epithelium. That this proliferating undifferentiated epithelium is capable of occasionally reverting back to the goblet cell type seems to lend support to the conception of the close relationship between the two types of cells, and might be considered as favoring the view that the undifferentiated epithelium

transition of normal goblet cells into undifferentiated non-mucin-containing cells. The increased power of proliferation following the loss of physiological function eventually leads to the formation of a stratified epithelium in glands of the intestinal adenoma.

Wechselmann<sup>15</sup> also concludes that there are two groups of intestinal adenomatous growths, one consisting of a hyperplasia of normal mucosal elements and the other composed of undifferentiated cells. He feels absolutely certain that the second group is due to abnormalities originating independently of catarrhal affections in accordance with Cohnheim's theory. He also gives evidence of a hereditary relation, citing cases where as high as six members of one family showed intestinal adenomatosis. He strongly opposes the etiological conception of susceptibility plus chronic inflammation. Oseki<sup>16</sup> found no evidence of inflammatory alterations in his material and therefore considers intestinal adenomas as neoplastic formations.

Ribbert's<sup>17</sup> views are similar to those of Cohnheim and Wechselmann. He found no indication of inflammation in the neighborhood of intestinal polypi and therefore opposes Verse's views regarding the part played by chronic catarrhal inflammation. Ribbert notes a sharp line of demarcation between the mucin and non-mucin-producing cells and opposes Verse's views supporting the transition of normal epithelium into undifferentiated non-mucin-producing cells found in adenomatous glands. The conception of disturbed development he believes is further supported by the frequent ability to trace polypi back to childhood. Borelius and Sjövall<sup>18</sup> attribute these newformations to an inflammatory process, although they concede the existence of an individual local disposition.

## CASE REPORTS

CASE 1. A female pig 9 month's old, weighing about 80 pounds, was submitted from a herd in which enteritis due to *Salmonella suispestifer* and coccidia had occurred frequently during the past. The only history available was that the pig had weighed more previously, showed diarrhea, and that it gradually became emaciated. At the time of autopsy the animal was in a poor state of nutrition. The haircoat was rough and dry, and the visible mucous membranes were pale. No evidence of a recent diarrhea was found. A hazelnut-



edematous fluid and infiltrated by leucocytes. The follicles were greatly enlarged and in nearly all instances the germ centers extended close to the periphery and showed endothelioid cell proliferation.

CASE 2. A female pig weighing about 100 pounds and presenting clinical symptoms of infectious enteritis was killed in a moribund condition. Infectious enteritis designates a form of enteritis produced by the *Salmonella suispestifer* as the primary factor and the *Actinomyces necrophorus* as a constant secondary invader.<sup>19</sup> The wall of the ileum was greatly thickened and congested. In most places the mucosa appeared raw and granular with floccules of caseated tissue firmly adherent to the surface. The cecum was slightly involved, while the anterior portion of the large colon was greatly thickened and in places covered by a diphtheric membrane. Incision through these areas revealed a heavy cellular structure which suggested a newgrowth in the mucosa. This condition diminished further back but the caseated membranes were still present. In addition to small areas of pneumonia and the presence of lung worms, the concomitant pathological changes usually found in other organs in infectious enteritis were present in this case.

*Microscopic Examination:* The tumefied portions of the wall of the ileum and large colon consisted of new-formed, adenomatous tissue. The glands were sometimes forked or showed numerous branches in contrast to the straight simple glands of the normal mucosa. In place of goblet cells the new-formed glands were lined by undifferentiated columnar epithelium similar to that described in the first case. At the edges of the adenomatous tissue there appeared transitions from one type of epithelium to another. In the deeper portions of some glands goblet cells were noted, while above was found a gradual or at times an abrupt change from goblet cells to undifferentiated cells. In the structures situated above the transitional areas the glands were lined by undifferentiated cells. Sometimes the newgrowth crowded out normal glands by pressure. In places, hypertrophic glands lined by goblet cells located above the muscularis mucosa were surrounded by adenomatous tissue. Evidence of an extensive mucosal destruction by a severe enteritis was noted in this case. In places the muscularis mucosa was involved by the retrograde process, although the adenomatous growth did not invade the deeper structures, the glandular proliferation taking

of polypi originates from normal gland epithelium which has undergone degeneration, rather than as originating from misplaced rudimentary cells. In the adjacent uninvolved parts of the intestinal mucosa the glands were lined by goblet cells which were often in a state of hyperactivity.

Cyst formation was common in the polypi. The epithelial cells lining the cysts were shorter and broader, the nuclei being less closely placed than in the tall columnar epithelium. The basement membranes of the new glands were intact and no evidence was found in any of the preparations of this case to suggest pathological malignancy, although the process must be considered as one of clinical malignancy. The pedicles of the polypi were short and consisted of well developed connective tissue structures emanating from the mucosa and submucosa, breaking up the muscularis mucosa and pulling the ends upward. The connective tissue portion of the pedicles divided, sending branches to different parts of the polypi to form a framework (Fig. 2). The mucosa and submucosa were often the seat of inflammatory processes, some of long duration. The adenomatous proliferation did not extend into the deeper structures. The lymph sinuses were distended.

Unlike the two subsequently described cases, which were associated with an active destructive enteritis process, this case showed a more or less definite line of demarcation between the new-formed tissue and the remnants of uninvolved mucosa. In this animal the process did not appear to spread progressively into the uninvolved area by the metaplasia of normal cells, further growth taking place by proliferation of the already degenerated epithelium, sometimes crowding out adjacent normal glands by pressure.

It was from such cases as this that some workers drew the conclusion that there can be no transformation of normal epithelium to this type as a result of destructive or inflammatory processes and that the undifferentiated cells originate from embryonic rudiments. The outer edges of the polypi often were involved by varying degrees of necrosis and covered by an exudate containing *Balantidium coli*. The location of the protozoa noted at this stage of the process did not suggest a correlation with the glandular epithelial proliferation.

The lymph nodes of the cecum and colon showed an advanced diffuse lymphadenitis. The trabecular tissue was separated by an

strands of fibroblasts or leucocytes. This case also showed the transitional pictures in which both goblet and undifferentiated cells appeared in the same gland identical with Case 2.

### EXPERIMENTALLY INFECTED CASES

This acute form of dysentery was reproduced in twelve pigs by feeding intestinal contents and scrapings from the mucosa of the cecum or large colon of naturally infected swine. The tissues of four animals from this experimentally infected group were studied microscopically. The same epithelial proliferative process described in previous cases was found in the cecum and large colon of these four animals.

### EPITHELIAL REGENERATION IN SEVERE INFECTIOUS ENTERITIS

During the course of our studies on the pathology of infectious enteritis in swine<sup>19, 20</sup> we sometimes observed in cases of extensive mucosal destruction histological pictures where the remaining epithelium of the intestine was given over entirely to the process of regeneration. From islands of altered columnar cells, remnants of a preëxisting normal mucosa, long strand-like cells stretched out over a denuded area in an attempt to cover it. The remnants of injured columnar cells from which the proliferation had its origin possessed large nuclei and an absence of mucin. The elongated proliferating cells reaching out into the denuded necrotic mucosa also had large oval nuclei and contained no mucin. The ability of such regenerated epithelium to repossess functional properties is an important point to consider in connection with diagnosis in unthrifty animals which have previously passed through a severe enteritis. Many such animals recovered and made considerable gain in weight, while others remained stunted. After the acute enteritis process had subsided the mucosa appeared thin and rough in places, readily distinguishable from a normal mucous membrane. An absence of goblet cells was often noted, together with epithelial regeneration. This fact, together with observations on adenomatous proliferations of the human intestine, would suggest that the manner of epithelial regeneration following the subsidence of an infectious or parasitic disease is of importance equal to the inflammatory and destructive

place chiefly in the upper mucosal structures. The surface of the newgrowth showed erosions and cellular infiltration, and was covered by a necrotic exudate which in places contained numerous *Balantidium coli*. Isolated glands contained single specimens of this protozoön.

CASE 3. On the following day another emaciated pig, weighing about 90 pounds, and showing symptoms of severe enteritis was killed in a moribund condition. The diarrheal excreta contained some blood. The usual alterations found in severe infectious enteritis were noted. The changes pertinent to this study were found in the large colon. In addition to areas showing hemorrhagic infiltration and diphtheric membrane some parts of the wall were greatly thickened. Like the previous case the increased thickness of the wall and the elevations of the mucosa consisted of a cellular structure suggesting a newgrowth.

*Microscopic Examination:* Microscopic study revealed a widespread destruction of the mucosa and extensive granulations often involving all structures above the inner circular muscle. The thickened parts of the large colon consisted of adenomatous proliferations, many of which were situated adjacent to ulcerated areas. The primary destruction involved the deeper structures of the wall as was evidenced by the extensive granulation tissue formation extending to the inner circular muscle. Under the adenomatous proliferations the submucosa was not completely destroyed. It is possible that in the latter areas some glandular tissue remained in the mucosa above from which regeneration and further proliferation could start, while in the ulcerated areas all epithelial or glandular structures were destroyed over a large area, permitting no chance for regeneration from any preëxisting epithelium. On the opposite edge of the ulcer the destructive process extended deeply, but left a portion of the submucosal structure above which glandular proliferation took place. Here the proliferative process had not advanced so far as in the first described field as shown by the smaller amount of newgrowth, small glands and less pronounced branchings. Goblet cells were also absent in this area. Characteristic branchings of glands in the more advanced parts of the adenomatous growths were found. As a rule the interglandular tissue was not so abundant in the newer parts of the proliferations near the lumen. Often the new-formed glands almost approximated, or were separated by only one or two

suggest a relationship between intestinal adenomatous proliferations and destructive processes followed by epithelial regeneration, as against their interpretation as independent tumors arising from isolated or misplaced embryonic rudiments.

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processes during the active stage of the disease. Owing to the short period of time elapsing before swine are killed for food it is possible for advanced adenomatous formations to pass by unnoticed, which may in part explain the rarity of its recorded presence in swine in contrast to the numerous reports of occurrence in the horse, cattle and man.

### SUMMARY

1. In experimental and field cases of infectious enteritis in which extensive mucosal destruction occurred, epithelial proliferation originating in the remaining islands of injured cells was noted. These proliferating cells were flattened and elongated with large nuclei. They stretched out into the necrotic tissue in an attempt to cover the denuded mucosa. Swine killed immediately after the subsidence of the infectious enteritis process usually showed an absence of goblet cells in the glands of the large colon, and in their place appeared either low or tall columnar epithelium with large nuclei.

2. An extensive destruction of mucosal tissue in two severe advanced cases of enteritis was associated with a degeneration of the epithelium and the formation of adenomatous growths. A transition from goblet cells to undifferentiated non-mucin-containing cells was found in some glands at the edges of the adenomatous tissue. In the advanced case showing adenomatous polypi, evidence of a previous inflammatory process was noted. Its etiology could not be determined from the microscopic study, although the herd history would suggest infectious enteritis. The blood serum of this subject agglutinated a *Salmonella suispestifer* antigen in a titre of 1:25. The rare presence of isolated goblet cells situated in large masses of new-formed glands lined by undifferentiated, non-mucin-containing cells is further evidence of a close relationship between the normal cells and those of the adenomatous growths. Newgrowths made up of glands lined entirely by goblet cells, described by some workers, were not encountered by us. The tumefied tissue consisted of glands lined by undifferentiated cells.

3. The characteristic bile duct proliferations in coccidiosis of the liver of rabbits, the adenomatous formations in the equine stomach due to nematodes, adenomas in bilharziosis and the characteristic columnar-cell-lined adenomatous proliferations described in connection with lung worm infestations and pneumonia of sheep <sup>21, 22</sup> all

## DESCRIPTION OF PLATES

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### PLATE 35

FIG. 1. Adenomatous polypi in the mucosa of the ileocecal valve, cecum and large colon. Case 1.

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PLATE 36

FIG. 2. Branching and cystic glands in the polypous growths from the cecum. Shows the connective tissue framework which emanates from the mucosal and submucosal connective tissue.  $\times 46$ .

Sand, soil or cinders are commonly ingested by swine. Particles of these become embedded in the exudate or mucosa and cannot be removed by washing. This accounts for the frequent tears found in the sections. It was often necessary to remove hard particles from the block while cutting.

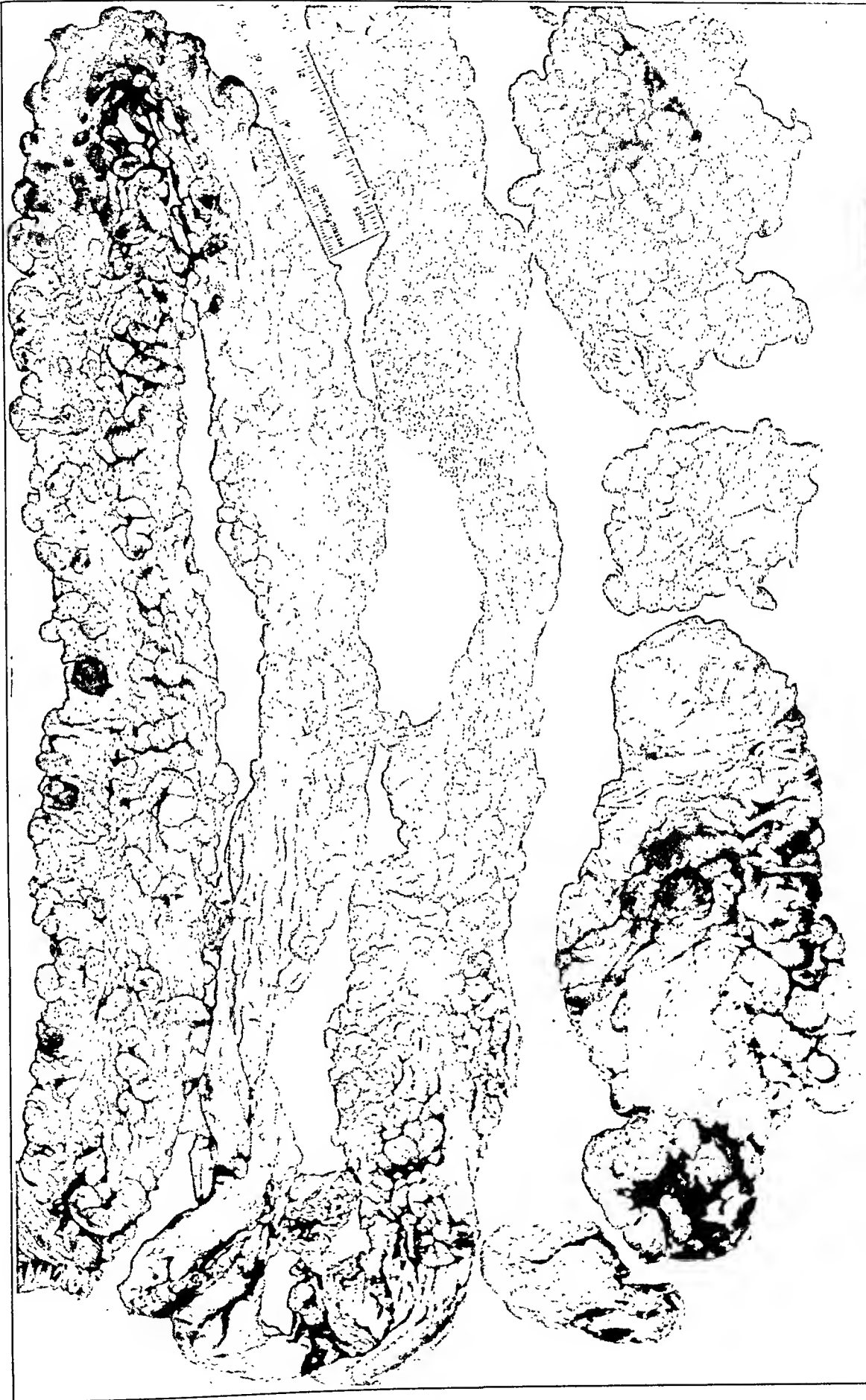
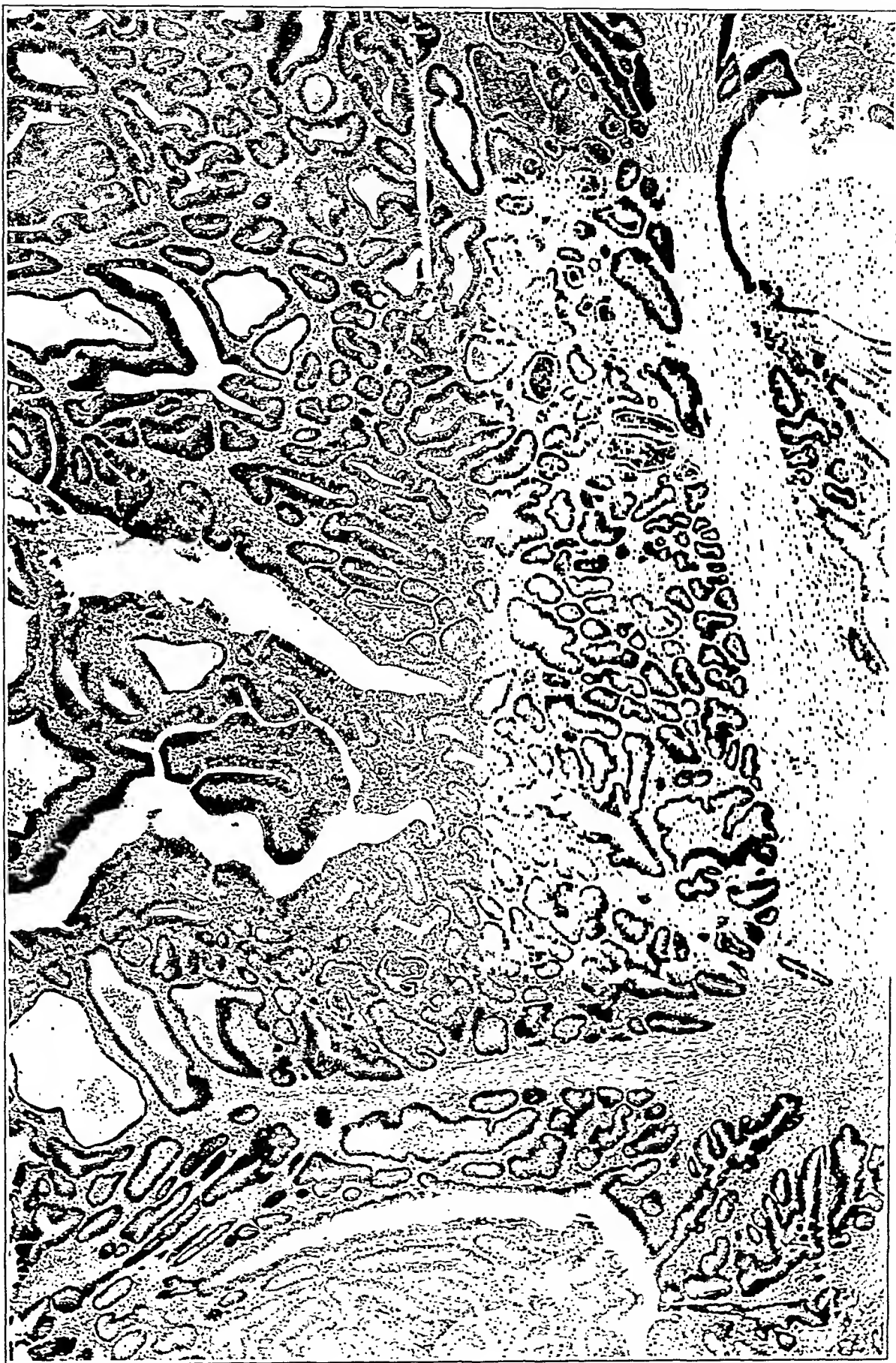


PLATE 37

FIG. 3. Large new-formed branching glands lined by undifferentiated columnar epithelium. The nuclei are closely packed and sometimes appear stratified. Goblet cells are absent.  $\times 110$ .







certain organs may be "predisposed" for the growth of malignant emboli; and Mallory (1914)<sup>2</sup> in referring to this problem says "Conditions of a chemical nature probably play an important part." Ewing (1928)<sup>3</sup> expresses the opposite view in the statement that "there is as yet no evidence that any one parenchymatous organ is more adapted than others to the growth of embolic tumor-cells."

I have been struck by the relative immunity from malignant metastases displayed by certain richly arterialised organs. In cases of systemic haematogenous dissemination of malignant tumours, the embolic influx into the various tissues of the body must be proportional to their respective arterial blood supplies, yet such richly vascular organs as the intestine, the spleen and the thyroid gland, are amongst the least frequent sites of development of secondary growths. Most noteworthy in this respect is the thyroid gland, which, next to the adrenal gland, is the most richly arterialised tissue in the body. The adrenal gland receives approximately 650 cc. of arterial blood per 100 grams of tissue per minute, and thrice this amount during a rise of blood pressure produced by adrenalin (Neuman, 1912).<sup>4</sup> The corresponding figure for the thyroid gland is only slightly less, namely 560 cc. per 100 grams per minute (Burton-Opitz, 1910).<sup>5</sup> With these high figures may be contrasted, for example, the relatively scanty arterial supply of the liver which receives only 26 cc. of arterial blood per 100 grams per minute (Burton-Opitz), or, according to Barcroft and Shore (1912),<sup>6</sup> 12 cc. for the resting liver and 23 cc. for the active liver subsequent to the digestion and absorption of food. Taking the total weights of the liver and thyroid gland to be 1500 and 25 grams respectively, it will be seen that the thyroid actually receives approximately one-half the volume of arterial blood received by the entire liver. Yet, while the liver is very frequently the seat of metastases from tumours of diverse kinds distributed in the systemic blood stream, metastatic growths in the thyroid gland are unusual. The striking disparity between the arterial vascularity and the incidence of metastatic neoplasms in thyroid tissue decided me to make a study of secondary tumours in this organ, with a view to determining, if possible, whether circulatory or chemical factors appeared to afford the better explanation of that disparity.

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## METASTATIC TUMOURS IN THE THYROID GLAND \*

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### I. INTRODUCTION

Malignant neoplasms of many kinds are distributed embolically in the arterial blood stream, and establish secondary tumours in various tissues. One of the most neglected problems of oncology is that of the preferential tissue affinities of the different types of malignant cell. Certain tissues and organs, for example the liver, are very frequent sites of metastases from neoplasms of very diverse kinds; while other tissues and organs, such as the skeletal muscles or the intestinal tract, are rarely the seat of malignant metastasis. Moreover, various tumours display decided predilections in the distribution of their metastases, notorious examples of this being the frequency of secondary growths in bones in cases of renal or of thyroid carcinomata. For the many vagaries of metastatic distribution observed in individual cases of malignant disease, no generally satisfactory explanations have been advanced. Various mechanical theories have been suggested, as for example that the movement of certain tissues inhibits the effective embolic lodgement of malignant elements; and the permeability of the capillaries of various tissues for minute solid particles has been much discussed. Other workers, however, have held that chemical rather than mechanical and circulatory factors play the principal rôle in determining the sites of development of metastatic growths. Thus Paget (1889)<sup>1</sup> speaks of certain tissues as being "congenial soil" for malignant growth, and views favourably the conception of Fuchs that

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frequently encountered, an opinion confirming that of Virchow to which reference has been made above. Kaufmann also mentions the case of a woman, aged 59 years, in whom an ovarian carcinoma produced minute metastases in an adenoma of the thyroid and in no other part of the gland.

*Naegeli* (1912)<sup>9</sup> records the case of an elderly female in whom an adenocarcinoma of the rectum produced metastases in thyroid adenomata. The thyroid had been the seat of long-standing irregular enlargement, and the cancerous deposits were most prominent in the degenerated adenomatous areas. A small probable metastasis in one lung was not examined microscopically.

*Kettle* (1912)<sup>10</sup> mentions a case in which he observed in an adenoma of the thyroid, a metastasis from a squamous carcinoma of the uterus.

*Rost* (1912)<sup>11</sup> describes the case of a man, aged 48 years, with "hypernephroma" metastases in the lungs, clavicle, thyroid and many lymph glands. The thyroid was much enlarged by long-standing goitrous changes, and contained also an area of secondary growth. The malignant elements were separated from the surrounding thyroid tissue by a wide zone of connective tissue, and from Rost's description it is apparent that the metastasis had become established in a fibrosed area of the goitrous gland.

*Müller* (1912)<sup>12</sup> describes a male patient, aged 64 years, who died with haemangiomatic growths in the gums, pleura, lungs, bowel, bones and thyroid gland. This organ was much enlarged: the right lobe was extensively calcified; the left lobe contained many adenomata, and in one of these, 2.5 cm. in diameter, there was an angiomatic nodule 8 mm. in diameter. While inclining to the view that the condition was a "system disease" involving the vessels of the various organs, Müller admits the possibility of metastasis from one of the tumours as a primary focus. Microscopically the growths presented the structure of cavernous angioma, with however, proliferation of the endothelial cells which in places were desquamating into the vascular spaces.

Most pathologists accept the neoplastic character of chloroma which therefore may be included legitimately in the present paper.

*Sauer* (1914)<sup>13</sup> describes a case of this disease in a male, aged 36 years, exhibiting in addition to other lesions, chloroma nodules in the thyroid, spleen, kidneys and prostate. The thyroid gland was

## II. REVIEW OF THE LITERATURE

The following résumé of recorded cases which exhibited metastatic tumours in the thyroid makes no claim to completeness. A reasonably careful search has been made of available pathological literature, but doubtless a number of isolated case reports in which thyroid metastases are recorded have escaped discovery. However, the material reviewed is adequate for the purposes of this paper, and the writer hopes that no important references on the subject have been omitted. A description is given first of those cases in which metastatic growths in the thyroid exhibited some noteworthy peculiarity, such as a special relationship to other pathological conditions of the gland. Then follows briefer reference to cases in which no details concerning the condition of the organ are recorded, or in which no striking peculiarity was observed.

*Virchow* in his book on tumours,<sup>7</sup> stated that malignant goitre might be either primary or secondary, that the latter might be either by direct extension from neighbouring growths or by metastasis from remote parts, and that cancerous or sarcomatous metastases might lodge in a thyroid gland already goitrous. *Virchow* exemplified such metastatic growths by two cases. The first case was that of a goitrous cretin, aged 53 years, who had had a testicular tumour removed eight years previously. At autopsy the old nodular goitrous thyroid contained a prominent tumour mass consisting of cells with large nuclei, and similar tumours were present in the lungs and sternum. Despite the long intervening period, *Virchow* regarded these several growths as metastases from the testicular tumour, and not as primary carcinoma of the thyroid. *Virchow's* second case was a female, aged 65 years, with a primary growth of the posterior pharyngeal wall. The thyroid gland, which exhibited diffuse colloid goitre, was the seat of two prominent metastatic tumours. Other secondary growths were present in the pancreas and kidney. In the same work, speaking of malignant melanotic tumours, *Virchow* also remarked of the less frequent sites of metastases that "the thyroid gland appears to be involved more frequently than the spleen."

*Kaufmann* in his *Lehrbuch* (*Reimann's* translation 1929, p. 537)<sup>8</sup> remarks the infrequency of metastatic neoplasms in the thyroid gland, and states his experience that melanotic growths are the most

Kaufmann (1902).<sup>25</sup>

(1) Malignant rhabdomyoma of prostate. Male, 26 years.

(2) Carcinoma of prostate. Male, 43 years.

Kaufmann (Lehrbuch, Reimann's translation, 1929).<sup>8</sup>

(1) Carcinoma of pharynx. Male, 62 years. (p. 824.)

(2) Adenocarcinoma of uterus. Female, 73 years. (p. 1681.)

Hirschfeld (1906).<sup>26</sup> Sarcoma of ala of ilium. Male, 36 years.

Grimm (1907).<sup>27</sup> Carcinoma of breast.

Offergeld (1909).<sup>28</sup> This author in a study of metastases from uterine cancer found records of seven cases with thyroid metastases. The original account is not immediately available to the present writer.

Davidsohn (1911).<sup>29</sup> Malignant melanoma of adrenal. Male, 58 years.

Chalier and Bonnet (1912).<sup>30</sup> Malignant melanoma of rectum. Male, 48 years.

Kettle (1912).<sup>10</sup> Squamous carcinoma of breast. Female, 69 years.

Mori (1913).<sup>31</sup>

(1) Malignant melanoma of eye. Male, 43 years.

(2) Carcinoma of breast. Female, 42 years.

(3) Carcinoma of breast.

Schöppler (1917).<sup>32</sup> Carcinoma of lung. Adult male.

Handley (1922).<sup>33</sup> Malignant melanoma of skin. Female, 34 years.

Jeannée (1925).<sup>34</sup>

(1) Carcinoma of bronchus. Male, 39 years.

(2) Malignant melanoma of skin. Female, 42 years.

(3) Carcinoma of bronchus. Male, 54 years.

Chajutin (1926).<sup>35</sup> Carcinoma of liver. Male, 60 years.

Derischanoff (1926).<sup>36</sup> Carcinoma of breast. Female, 58 years.

di Biasi (1926).<sup>37</sup>

(1) Squamous carcinoma of breast. Female, 47 years.

(2) Carcinoma of breast. Female, 48 years.

(3) Naevus carcinoma of skin. Male, 52 years.

(4) Carcinoma of breast. Female, 69 years.

(5) Malignant melanoma of adrenal region. Male, 83 years.

(6) Carcinoma of breast. Female, 73 years.

(7) Carcinoma of breast. Female, 60 years.

(8) Carcinoma of lung. Male, 52 years.

In two other cases described by di Biasi, metastases recorded in the thyroid were queried, these two cases therefore are excluded.

White and Brunton (1927).<sup>38</sup> Brief reference is made to two cases in which it is stated that thyroid metastases were present, but information given is inadequate to justify inclusion.

Brandt (1927).<sup>39</sup> Carcinoma of kidney. Female, 75 years.

Girdwood (1929).<sup>40</sup> Malignant melanoma of skin. Female, 42 years.

If we regard Hodgkin's "granuloma" as neoplastic in nature, involvement of the thyroid in that disease also falls in the scope of this paper. Osler (1885)<sup>41</sup> mentions two cases of thyroid enlargement due to deposits of "Hodgkin's tissue," but gives no details. Beitzke (1909)<sup>42</sup> described a case of Hodgkin's disease in a woman, aged 65 years, in which the lymph glands of the upper parts of the

decidedly enlarged, and contained a number of well defined greenish nodules in both lobes. These proved to be thyroid adenomata infiltrated by chloroma cells which extended thence into the adjacent, more normal thyroid tissue. Sauer could discover only one other previously recorded case of chloromatous deposits in the thyroid gland, described by *Pribram* (1909)<sup>14</sup> in a male, aged 22 years.

*Herxheimer* (1925)<sup>15</sup> describes diffuse myeloblastic infiltration of the thyroid in a case of leukaemia.

*Reinhart* (1917)<sup>16</sup> records the case of a female patient, aged 38 years, who suffered from carcinoma of the left lung with metastases in the right lung, pleura, kidneys, adrenals, liver, thyroid, lumbar spine, and thoracic and retroperitoneal lymph glands. In the right lung and in the bronchial glands, chronic tuberculosis coexisted with the malignant disease. The thyroid, only slightly enlarged, contained several metastatic nodules of growth, and was also the seat of tuberculous disease with fibrosis, lymphocyte accumulation, and numbers of tubercles. Reinhart remarks on the unusual combination of cancerous metastasis and tuberculous disease of the thyroid which is an infrequent site of either of these conditions, and suggests the possibility of a causal relationship of the *locus minoris resistentiae* kind.

*Prym* (1924)<sup>17</sup> records a case of chorionepithelioma in a woman, aged 44 years. Metastases were present in the lungs, brain, liver, bowel mucous membrane, both kidneys and thyroid gland. This last organ was the seat of multiple adenomata, and the metastatic deposit was located within one of the adenomatous areas.

In the above cases the striking relationship of the metastatic growths in the thyroid gland to other abnormalities necessitated special comment. In many other cases no such noteworthy associations are recorded, or, more often, no details whatever are available concerning the condition of the organ. It is not necessary, therefore, to describe these cases individually, and the abbreviated information given in the following list will suffice.

Foerster (1858).<sup>18</sup> Carcinoma of cervix uteri.

Rosenblatt (1867).<sup>19</sup> Carcinoma of liver.

Blau (1870).<sup>20</sup> Carcinoma of corpus uteri.

Beck (1884).<sup>21</sup> Carcinoma of lung. Male, 65 years.

Siegel (1887).<sup>22</sup> Carcinoma of lung. Female, 68 years.

Ehrich (1891).<sup>23</sup> Carcinoma of lung. Female, 52 years.

Kantorowicz (1893).<sup>24</sup> Carcinoma of breast. Female, 51 years.

metastases in the liver, spleen, kidneys, adrenals, pancreas, dura mater, several ribs and thyroid gland. The last named organ was small and of normal external appearance, but on section a metastasis 1 cm. in diameter was found in the upper pole of the right lobe (Fig. 1), and two smaller separate nodules 3 mm. in diameter near the isthmus. The thyroid tissue throughout was pale and tough and exhibited irregular strands of fibrous tissue. Subsequent microscopic study revealed extensive fibrosis, parenchymatous atrophy, and plentiful accumulations of lymphocytes.

*Histological Findings:* Anaplastic polyhedral and signet-celled carcinoma, in parts exhibiting loose alveolar formation, in parts diffuse; scanty suggestions of adenomatous grouping; mitoses plentiful; stroma scanty; much degenerative change (Fig. 2).

CASE III. *Clinical History:* Female, 61. January 1929, patient noticed a small lump in left breast. June 1929, paresis of left limbs appeared, with spasticity and extensor plantar reflex on left side. Thereafter, rapid emaciation and asthenia, mild convulsive spasms of left arm, and aphasia. Death in August 1929.

*Autopsy Findings:* Non-ulcerating mass 5 cm. in diameter in left breast, adherent to skin and invading pectoral muscles; tissue partly dense and hard, partly soft and mucoid. Apical axillary and supra-clavicular glands involved with extension to upper two ribs and intercostal spaces. Numerous small nodules of growth beneath visceral and parietal pleura on both sides; a few metastases in lung substance. Metastases also present in liver, left kidney, both adrenals, dura mater, cerebrum and the thyroid gland. This organ was somewhat enlarged and exhibited irregular colloid retention and many small adenomata and cysts. It contained a solitary metastasis 1 cm. in diameter in the right lobe near the isthmus (Fig. 3).

*Histological Findings:* Primary tumour an adenocarcinoma with extensive areas of mucoid change. Metastases similar, but less prominently adenomatous and more often carcinoma simplex in type (Figs. 4 and 5).

CASE IV. *Clinical History:* Female, 70. A firm mass the size of a walnut in left breast for 30 years. Early in 1928 this began to enlarge. December 1929, rapid growth of mass with cough and dyspnoea. By January 1930, mass was a firm hemispherical tumour 10 cm. in diameter projecting anteriorly, adherent to skin but not to muscles or chest wall. Death in February 1930.

body were involved, and tumour-like masses were present in the bone marrow, skull periosteum, liver and thyroid gland.

Finally, mention must be made of three cases referred to by Ewing (1928, p. 961),<sup>3</sup> namely Pick's and Ehrhardt's cases of osteosarcoma and Fraenkel's case of melanosarcoma. As the original reports are not immediately accessible, and as there appears to be doubt as to whether the tumours in the thyroid were primary or secondary, I have excluded these cases from further consideration in this paper.

### III. PERSONAL OBSERVATIONS

The following examples of metastatic tumours in the thyroid gland have been observed by the writer in personally conducted autopsies. The descriptions are abbreviated, but all essential details are included.

*CASE I. Clinical History:* Male, 47. In 1923, left eyeball excised for pigmented intra-ocular tumour. Good health thereafter for over four years. July 1927, recurrence of tumour in orbital cavity, and clinical enlargement of liver. Death in February 1928.

*Autopsy Findings:* Recurrent melanotic tumour of orbit. Numerous partly pigmented, partly colourless metastases in the myocardium, endocardium, right lung, liver (280 ounces), kidneys, both adrenals, retroperitoneal tissues and thyroid gland. The latter, otherwise normal, contained a solitary pigmented metastasis 1.5 cm. in diameter in the left lobe.

*Histological Findings:* Tumour cells, both polyhedral and fusiform, arranged both diffusely and in discrete clumps in relatively scanty stroma. Pigment formation varies in degree in different areas, in places absent, in places massive, both intra- and extra-cellular in situation.

*CASE II. Clinical History:* Female, 54. January 1927, "indigestion" appeared. June 1927, radical excision of rectum after preliminary colostomy. Good health thereafter for nearly two years. April 1929, pain in right leg and some difficulty with micturition; vaginal examination revealed large hard mass occupying right side of pelvic cavity. Death in September 1929.

*Autopsy Findings:* Large firm cancerous mass in pelvic cavity, adherent to sacrum and right pelvic wall, and infiltrating perineum and lower part of uterus. A moderate number of small scattered

steadily enlarged. Haematuria continued; fibrinuria also present, the urine sometimes being very viscid and almost gelatinous. Skiagrams revealed erosion of the right ilium. Died July 1930.

*Autopsy Findings:* Large tumour of left kidney (28 ounces) with gross invasion of renal vein and projection of tumour into inferior vena cava. Metastases in lungs, liver, pancreas, thyroid gland, ribs and ilium. The caudal pole of the right lobe of the thyroid contained an irregular haemorrhagic metastasis 2 cm. in diameter with several neighbouring but connected satellite nodules (Fig. 9).

*Histological Findings:* Typical clear-celled papillary carcinoma of the kidney (Fig. 10). The thyroid tissue exhibited some general fibrosis, vascular degeneration and pigmentation, but these changes were not excessive for the patient's age.

CASE IX. *Clinical History:* Female, 56. January 1929, pain and lump noticed in left breast; radical amputation in April; carcinoma simplex with axillary glands involved. September 1929, recurrence in right breast; amputation; carcinoma simplex; subsequent local recurrence and signs of intrathoracic extension. Died October 1930.

*Autopsy Findings:* Extensive nodular infiltration of both pectoral regions, with invasion of intercostal spaces and extensive involvement of pleurae and thoracic lymph glands. Metastases in liver, adrenals, peritoneum, abdominal and cervical lymph nodes, dura and thyroid. The thyroid was small, tough and poorly vesicular, and contained eight to ten metastatic nodules up to 5 mm. in diameter, as well as several diffuse areas of infiltration.

*Histological Findings:* Small spheroidal-celled carcinoma simplex. The thyroid metastases occurred both as localised nodules and as diffuse infiltrations (Fig. 11). The thyroid tissue itself was the seat of advanced fibrosis, parenchymatous atrophy and irregular adenoma formation with distorted irregular vesicles poor in colloid. Several of the metastatic nodules lay in the centres of adenomatous areas.

CASE X. *Clinical History:* Male, 54. April 1930, noticed small nodule in right ear near meatus. A month later mass appeared in neck just below ear. By June the ear nodule had become an ulcer 1.5 cm. in diameter on the inner aspect of the pinna, and there was a hard mass of glands in the upper cervical region. Diathermy of the ulcer given and radium needles buried in glands. Temporary improvement, but died in October 1930.

*Autopsy Findings:* Mammary tumour restricted to fat and not involving pectoral muscles; axillary glands not enlarged. Numerous large metastases in both lungs and in parietal pleurae and mediastinal glands. A few small nodules in the liver. Thyreoid, of normal size, contained several adenomata up to 1 cm. in diameter; and the left lobe presented two tiny firm white nodules which proved microscopically to be metastases. These were not situated in adenomatous tissue.

*Histological Findings:* Anaplastic spindle and polyhedral-celled carcinoma, largely diffuse but partly alveolar in arrangement; numerous mitoses; scanty stroma.

CASE V. Female, 58. (Reported in detail elsewhere.)<sup>43</sup> Anaplastic carcinoma of breast; thyreoid almost totally replaced by metastatic growth; clinical symptoms of myxoedema.

CASE VI. Female, 41. (Reported in detail elsewhere.)<sup>44</sup> Malignant sacral chordoma with widespread metastases. The thyreoid gland was enlarged to double its normal size and was the seat of prominent fibrosis and many adenomata. Several chordoma metastases were present, the largest 8 mm. in diameter (Fig. 6). Several of these lay within adenomatous areas. Microscopically the thyreoid tissue exhibited extensive fibrosis, parenchymatous atrophy and lymphocytic infiltration (Fig. 7).

CASE VII. *Clinical History:* Female, 53. September 1929, post-prandial epigastric pain and loss of weight. December, laparotomy revealed inoperable gastric cancer. Death in March 1930.

*Autopsy Findings:* Extensive gizzard carcinoma of the stomach with metastases in the coeliac and upper lumbar lymph nodes, the liver, peritoneum, ribs, sternum, skull and thyreoid. Microscopic tumour emboli were found also in the lungs. The thyreoid gland was small, pale and tough, and contained a single metastatic nodule 6 mm. in diameter.

*Histological Findings:* A disorderly adenocarcinoma. The metastasis in the thyreoid (Fig. 8) consisted of irregular acini of columnar-celled carcinoma set in an abundant, partly hyalinised fibrous stroma, which extended into the adjacent thyreoid tissue where cystic and calcareous changes were also present.

CASE VIII. *Clinical History:* Male, 73. Intermittent haematuria began in 1925. Pain in right loin and sacro-iliac region began in 1927. A swelling appeared in the latter situation in 1928. This



ten cases, nine occurred in a series of 170 consecutive autopsies on unselected malignant cases of all kinds, the incidence of metastases in the thyroid thus being 5.2 per cent. Admittedly no statistical deductions can be made from so small a series of cases; nevertheless I attribute this relatively high incidence of thyroid deposits at least in part to the fact that the gland in each case was dissected out and thoroughly sectioned, a procedure not always adopted in routine autopsy work. Had this not been done, the metastases present in Cases II, IV, VII, IX and X could readily have eluded discovery. From personal experience, I am satisfied that mere bilateral section of the thyroid in situ, a procedure frequently adopted, is an inadequate examination of the organ.

#### INFLUENCE OF TUMOUR-TYPE AND ORIGIN ON FREQUENCY OF THYROID METASTASES

Little reliable information can be obtained from the literature regarding the relative incidences of thyroid metastasis from various

	Cases
Carcinoma of breast .....	15
Malignant melanoma .....	9
Carcinoma of bronchi and lungs .....	8
Carcinoma of uterus .....	4
Carcinoma of kidney .....	3
Chloroma .....	2
Carcinoma of liver .....	2
Carcinoma of rectum .....	2
Carcinoma of pharynx .....	2
Carcinoma of stomach .....	1
Carcinoma of testis .....	1
Carcinoma of ovary .....	1
Carcinoma of prostate .....	1
Malignant rhabdomyoma of prostate .....	1
Carcinoma of skin of ear .....	1
Haemangio-endothelioma .....	1
Malignant sacral chordoma .....	1
Choriocarcinoma .....	1
Sarcoma of bone .....	1
Total .....	57

individual tumour groups. Kaufmann's impression that the incidence is relatively high in the case of melanotic growths receives statistical confirmation from the figures given by Eiselt (1861),<sup>47</sup> who, in 50 autopsies on such cases, found an incidence of 12 per

*Autopsy Findings:* Nodular recurrence in caudal wall of meatus, and large infiltrating mass in neck. Internal jugular vein obliterated and invaded and contained friable tumour thrombus. Massive metastases in lungs and thoracic lymph glands. A few small metastases in left kidney, right rectus abdominis muscle, mesentery, inguinal glands of both sides, and thyroid. This last organ, otherwise substantially normal, contained two small white nodules each 4 mm. in diameter.

*Histological Findings:* Highly anaplastic non-cornifying epidermoid carcinoma with many mitoses, many giant tumour cells and frequent diffuse arrangement. The white nodules in the thyroid proved to be small spherical adenomatous areas containing infiltrating metastatic tumour deposits (Fig. 12).

#### IV. DISCUSSION AND DEDUCTIONS

##### THE FREQUENCY OF METASTATIC GROWTHS IN THE THYROID GLAND

The numerical incidence of secondary tumours in any given organ is difficult to assess with accuracy, and this difficulty is greater in the case of certain organs which unfortunately are not always examined at autopsy with the same thoroughness as the major viscera. In many collected statistics which contain valuable figures concerning the incidence of metastatic growths in the lungs, liver, spleen and other principal organs, one feels less confident of the information relating to such viscera as the testis, the pituitary body or the thyroid. Probably the German and other continental pathologists are the least fallible in this respect, and the following figures given by Müller (1892)<sup>45</sup> may be cited. In 521 autopsy cases of carcinoma, in which metastases were present in 47.2 per cent., the thyroid gland was involved in 1.5 per cent. In 102 cases of sarcoma, 63.7 per cent. of which exhibited metastases, the thyroid was involved in 3.1 per cent. Kitain (1922),<sup>46</sup> in a statistical study of 452 autopsies on cases of cancer, found the thyroid gland the seat of metastases in 14 (*i. e.*, 3.1 per cent.), the sites of primary growth being breast 8, skin 1, larynx 1, pancreas 1, pharynx 1, thymus 1, lung 1.

My own experience suggests that the thyroid is more frequently the seat of metastatic deposits than is generally recognised. Of my

existed with a metastasis from a lung carcinoma; and the author suggested that the presence of the tumour predisposed the gland to tuberculous infection. From Reinhart's description, however, it is apparent that the tuberculous disease in the thyroid was not recent, exhibiting well developed tubercles and areas of fibrosis, and it is improbable that the multiple nodules of secondary growth antedated these chronic inflammatory changes. If, then, a causal relationship existed between the two processes, it is more probable that the tuberculous changes predisposed the organ to the establishment of the metastatic tumours, an interpretation the converse of that suggested by Reinhart.

In my own material, preëxisting abnormalities of the thyroid tissue were a prominent feature. In Case II the gland exhibited extensive fibrosis and lymphocyte accumulation; in Cases III and VI, adenomatous, cystic and fibrotic changes were present throughout the organ, and several of the smallest chordoma metastases were situated within adenomatous areas. In Case VII the metastatic growth was located in an area of tissue which was the seat of old fibrosis and calcification. In Case IX the thyroid tissue exhibited advanced fibrosis and adenomatous changes, and several of the metastatic nodules occupied adenomatous areas. In Case X both of the small metastases present lay within adenomata. Thus, in no less than six of my ten cases the metastatic growths present either exhibited a decided preference for adenomatous or fibrosed areas of thyroid tissue, or else occurred in an organ which was the seat of universal retrograde changes decidedly exceeding normal limits. From the evidence afforded by these observations and with the several striking instances cited from the literature, I cannot avoid the conclusion that the normally slight susceptibility of thyroid tissue to the development of metastatic growths is decidedly augmented by the presence of adenomatous and other retrograde structural changes in that tissue.

What factors might determine this predisposition of altered thyroid tissue to metastatic growths? Two possibilities present themselves, (*a*) altered vascular conditions in adenomatous or other abnormal areas may favour the arrest of blood-borne emboli; or (*b*) structurally altered thyroid tissue may be a chemically more favourable soil than normal tissue for the development of secondary neoplasms. These alternative hypotheses will be discussed briefly.

cent. This figure much exceeds the estimates cited above for malignant tumours in general.

Further information on the relationship between the type and origin of the primary neoplasm and the frequency of thyroid metastases may be obtained by a tabulation of the origins of the 57 tumours reviewed in this paper.

The high place occupied by carcinoma of the breast is doubtless due to the great frequency of that disease. Yet it is noteworthy that an almost equally frequent neoplasm, gastric carcinoma, is poorly represented. Melanoma and lung carcinoma, both relatively uncommon tumours, take second and third places on the list. Lung cancer, which is recognised as possessing an unusual tendency to metastasise to the brain and adrenal glands (Dosquet, 1921),<sup>48</sup> evidently exhibits also a similar predilection for the thyroid. The position occupied by melanoma in the table confirms the relatively high incidence of thyroid metastases in this disease remarked by Kaufmann and by Eiselt.

#### THE RELATION OF METASTASES TO PREEXISTING ABNORMALITIES OF THE THYROID

Frequently, records of secondary growths in the thyroid give no detail regarding the condition of the thyroid tissue. In a number of cases, however, in which structural details are recorded, the association of the metastatic deposits with areas of abnormal thyroid tissue has been very striking. Attention is again directed to those cases which received special comment early in this paper. In Virchow's case of testicular tumour, the only sites of metastasis beyond the lungs were the sternum and an old nodular goitrous thyroid. In Kaufmann's case of ovarian carcinoma, in Naegeli's case of rectal cancer, in Kettle's case of uterine carcinoma, in Sauer's case of chloroma, and in Prym's case of chorionepithelioma, the metastatic growths in the thyroid were located in and restricted largely to adenomata, while the more normal thyroid tissue was uninvolved. In Rost's case of "hypernephroma," a metastasis occupied an area of fibrous tissue in an old goitrous thyroid. Accepting the metastatic character of Müller's case of haemangio-endothelioma, the metastasis in the thyroid lay within an old fibrous adenoma. In the case described by Reinhart, chronic tuberculous thyroiditis co-

sues are poor soils for malignant growth partly because of their high oxygen tension, while poorly oxygenated organs like the liver offer a metabolically more favourable nidus for the proliferation of arrested cancer cells. Now adenomatous and other retrograde changes result in atrophy of the thyroid parenchyma and its replacement by poorly vascular fibrous tissue, and the increased vulnerability of adenomatous or fibrosed areas to malignant metastasis may be due to the partial loss of those parenchymatous and metabolic qualities which antagonise neoplastic development.

Some support for this hypothesis is afforded by consideration of the tumour types most frequently responsible for thyroid metastases. Our review of 57 neoplasms has shown that, next to mammary cancer (15 cases), two relatively rare forms of malignant growth, melanoma (9 cases) and lung cancer (8 cases), were the most potent in producing metastatic deposits in the thyroid gland, while many commoner neoplasms, *e. g.*, the alimentary carcinomata, are relatively ineffective in this respect. These observations strongly suggest that some metastasising neoplasms, notably melanoma and lung carcinoma, manifest a decidedly greater inherent capacity than others for colonising the thyroid. It is difficult to see how the different incidence of thyroid metastasis by different tumour-types can depend on any local vascular conditions in the organ. That it is determined rather by a variable metabolic relationship between thyroid tissue and the various types of malignant elements which effect lodgement therein, appears much more probable. Some kinds of tumour cells, *e. g.*, those of melanoma suffering embolic arrest in the thyroid gland, find their chemical environment suitable to continued extravascular multiplication, while to other kinds of malignant cells thyroid tissue is an uncongenial soil, in which, therefore, these cell deposits become sterile. This sterility, however, is only relative, and continued proliferation of the malignant cells may ensue should they chance to lodge in an adenomatous or other abnormal area of the gland where retrograde processes have deprived the tissue somewhat of its inhospitable metabolic qualities.

Another striking point emerges on further consideration of those recorded cases in which thyroid metastases have exhibited some notable association to other lesions of the organ. The responsible tumours in these cases were carcinoma of testis (Virchow), carcinoma of ovary (Kaufmann), carcinoma of rectum (Naegeli), carcinoma of

In support of the first hypothesis might be advanced the observations of Simpson (1913),<sup>49</sup> Monogenow (1913)<sup>50</sup> and others on the impoverished blood supply to thyroid adenomata. The vascularity of adenomatous tissue is much less than that of normal thyroid, and the vessels exhibit all grades of degenerative change. It might be argued, then, that the relatively poorly vascularised adenomata with their deteriorated vessels lack the "flushing" action obtaining in normal tissue, so that the effective lodgement of minute emboli is favoured. On the other hand, since the embolic influx into any tissue must be proportional to its blood supply, the diminished vascularity of adenomata necessarily reduces the chances of malignant fragments entering these parts of the organ. Hence while it is *possible* that vascular conditions in adenomatous thyroid tissue may favour embolic arrest, it is also *certain* that these same conditions minimise the opportunities of embolic entry. Further, it is entirely an assumption that malignant emboli are in general so minute that differences in capillary calibre or velocity of blood flow in different tissues will influence to any great degree the chances of embolic lodgement. We know that the average diameter of the capillaries in various tissues is approximately equal to the diameter of a single red blood corpuscle and that the highly plastic red corpuscles frequently suffer great deformation in traversing the narrower capillaries (Krogh, 1929).<sup>51</sup> Malignant embolic fragments must be much less deformable than blood corpuscles, and their size must usually be much larger than that of a single erythrocyte; often, indeed, neoplastic emboli certainly consist not of single cells but of clumps of cells or of fragments of thrombus bearing malignant cells. Such fragments must certainly suffer arrest in the arterioles or capillaries of any tissue. For these reasons therefore it is improbable that vascular deterioration is the principal factor determining the different incidence of metastatic tumours in healthy and in altered thyroid tissue.

The second hypothesis, that altered thyroid tissue is more suitable than normal tissue as a nidus for malignant growth, is suggested by (a) the peculiar characters of the thyroid parenchyma, which, with its large content of iodine-rich colloid, is a chemically unique tissue, and by (b) the high oxygenation of that tissue. Malignant cells have a relatively anaerobic metabolism (Warburg, 1930).<sup>52</sup> It is possible then that the thyroid and other well arterialised tis-

tive and precisely opposite interpretation. Practical medicine recognises the curative influence of iodine in mycotic infections, and it is possible that in Benelli's case the mycotic organism found the iodine-deficient adenomatous tissue a more favourable soil than the normal iodine-rich parenchyma. This case indeed may be interpreted as actually illustrative of the biochemical conception of tissue predilection for metastatic development.

For the various reasons outlined above, I believe that the frequent association of secondary tumours in the thyroid with other abnormalities of the organ is not to be explained as merely coincidental, but that structurally altered thyroid tissue is indeed predisposed to metastatic developments, and that this predisposition depends on the chemical or metabolic qualities of the recipient tissues with respect to the requirements of malignant cells arrested therein. We return then to the view of Fuchs and Paget mentioned in our introduction. To adopt Paget's simile, malignant seed, though necessarily sown less lavishly in the altered than in the normal tissue, germinates more readily in the former.

#### ENDOCRINE DISTURBANCES OCCASIONED BY METASTASES IN THE THYROID

Thyroid deficiency resulting from neoplastic destruction of the thyroid gland is exemplified by my Case V, reported in detail elsewhere. A remarkable observation made first by Hirschfeld (1906)<sup>26</sup> and later by Mori (1913)<sup>31</sup> is that exophthalmos, tachycardia, tremor and other thyreotoxic symptoms may result from a metastatic tumour in the thyroid gland; Ewing (p. 949)<sup>3</sup> has observed the same event. In three such cases Mori found this organ to be the seat of colloid goitre with plentiful deeply staining secretion; while in another case of metastasis in the thyroid without thyreotoxic symptoms the gland was poor in colloid. Mori concluded that the thyreotoxic state was due to excessive absorption of secretion from active thyroid tissue subjected to mechanical pressure by the enlarging metastasis and its stroma. This conclusion appears to me to be based on inadequate data, and not to be supported by the observations of other writers. In many of the other cases reviewed in this paper colloid goitrous changes were recorded, and in my own material abundant colloid-rich tissue was present in Cases III, VI and VIII, yet no thyreotoxic symptoms had been present.

uterus (Kettle), chloroma (Sauer), carcinoma of lung (Reinhart), haemangio-endothelioma (Müller), "hypernephroma" (Rost), chorionepithelioma (Prym), carcinoma of rectum (my Case II), carcinoma of breast (Case III), chordoma (Case VI), carcinoma of stomach (Case VII), carcinoma of breast (Case IX) and carcinoma of skin (Case X). Observe that in these 14 cases, carcinoma of the lung is represented only once and melanoma not at all, despite our finding that, next to mammary cancer, these two forms of tumour are most frequently responsible for metastases in the thyroid. It is notable in this respect that the only one of our own seven cases in which the thyroid parenchyma was entirely healthy was one of melanoma, and this was also the case in other recorded examples of melanoma in which details are given concerning the thyroid gland, those of Chalié and Bonnet, Mori, and Girdwood. Evidently then, those very tumour-types which possess a maximum propensity for colonising thyroid tissue seldom exhibit any remarkable association with other abnormalities of that tissue; while, on the contrary, such notable associations are encountered frequently in the case of other growths which seldom produce metastases in the thyroid. This strongly suggests that these latter growths find healthy thyroid tissue an infertile nidus and hence tend to remain sterile therein, while such infertility is less in adenomatous and other diseased areas, which therefore are frequently present in association with metastases from neoplasms of small thyroid-colonising capacity. These considerations confirm the predisposition of altered thyroid tissue to metastasis, and support the metabolic rather than the vascular hypothesis regarding this predisposition.

In pondering these problems, it occurred to me that a study of other non-neoplastic embolic processes in the thyroid tissue might shed light on the questions under discussion. Accordingly a search of the literature for records of pyaemic and other metastatic inflammatory lesions of the gland was made. Only one reference to the association of such a lesion with a thyroid adenoma was discovered. Benelli (1912)<sup>53</sup> described a case of mycotic infection of the gastric mucous membrane. The thyroid gland contained a metastatic mycotic abscess, and this was located within a thyroid adenoma. This observation might appear at first to support the vascular rather than the metabolic hypothesis of the predisposition of adenomata to metastatic processes, but further consideration suggests an alterna-



cer) notable association of the metastases with other abnormalities of the gland are observed only infrequently. Conversely, thyroid metastases from growths of low thyroid-colonising tendency exhibit remarkably frequent association with preëxisting abnormalities of the organ.

NOTE: I am indebted to Professor P. MacCallum of the Department of Pathology, Melbourne University, for his stimulating criticism and interest, and to members of the Honorary Staffs of the Austin and Alfred Hospitals, Melbourne, for their consent to my utilising the histories of the cases.

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## CONTIGUITY-INVASION OF THE THYROID GLAND

Though strictly not falling within the scope of this paper, it is desirable to refer briefly to the phenomenon of direct invasion of the thyroid gland by contiguous neoplasms. Primary tumours of the pharynx, larynx, oesophagus or thymus may infiltrate the thyroid, and this event is a frequent finding in cases of extensive cervical glandular metastases from carcinoma of the lip, tongue and pharynx. In a series of 35 autopsy cases of epidermoid carcinoma of the head and neck <sup>54</sup> I found the thyroid invaded by growth in nine cases. In one remarkable case of this kind <sup>55</sup> the extensive cervical metastases from a lingual cancer had almost completely destroyed the gland, and compensatory hyperplasia of an accessory lingual thyroid had occurred.

Primary endothelioma of the cervical lymph nodes may invade the thyroid gland, as in Flournoy's case (1907)<sup>56</sup>; and the same may be observed in Hodgkin's disease of the cervical region (Osler, 1885).<sup>41</sup> The writer has seen an enormous anaplastic carcinoma of the parotid gland, the peripheral extensions of which infiltrated the thyroid; and malignant intrathoracic neoplasms may extend into the neck and directly invade this organ (Jacobs, 1927).<sup>57</sup>

## SUMMARY AND CONCLUSIONS

1. Forty-seven collected records of metastatic growths in the thyroid gland are reviewed, and ten personal cases are added and described.

2. Secondary tumours occur more frequently in the thyroid than is generally recognised, and a plea is made for more thorough pathological examination of this organ in cases of malignant disease.

3. There are good grounds for believing that different types of tumours possess different intrinsic capacities for establishing metastases in the thyroid, and that melanoma and lung carcinoma are the most potent in this respect.

4. There is strong evidence that adenomatous and other abnormal areas of thyroid tissue are predisposed to the establishment of metastatic neoplasms, and that this predisposition depends on chemical or metabolic rather than on vascular changes in the altered tissues.

5. In the case of those neoplasms which display a maximum propensity for metastasising to the thyroid (melanoma and lung can-

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## DESCRIPTION OF PLATES

### PLATE 38

- FIG. 1. Case II. Vertical sagittal section of the right lobe of the thyroid showing the metastatic growth at the upper pole (A). The fibrotic condition of the gland elsewhere is evident. (Natural size.)
- FIG. 2. Case II. Photomicrograph of the metastasis shown in Fig. 1. A clump of loosely aggregated anaplastic tumour cells, some of signet ring type, are seen amidst thyroid parenchyma which exhibits lymphocyte accumulation. × 180.
- FIG. 3. Case III. Two sagittal sections of the right lobe of the thyroid, showing the infiltrating metastatic growth, indicated by arrows. Observe the advanced adenomatous and cystic changes in the gland elsewhere. (Natural size.)
- FIG. 4. Case III. Photomicrograph of the metastasis shown in Fig. 3. Mucoid adenocarcinoma is seen invading thyroid tissue, which exhibits fibrosis, distortion of vesicles and aggregation of lymphocytes. × 80.
- FIG. 5. Case III. Another area of the same growth, showing infiltrating carcinoma simplex. × 180.
- FIG. 6. Case VI. Vertical sagittal section of the left lobe of the thyroid, showing two translucent chordoma nodules denoted by arrows. Notice the conspicuous adenomatous, cystic and fibrous changes throughout the gland. (Natural size.)

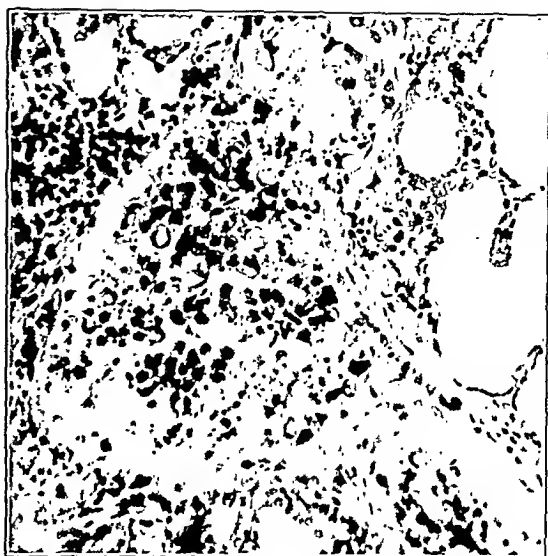
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PLATE 39

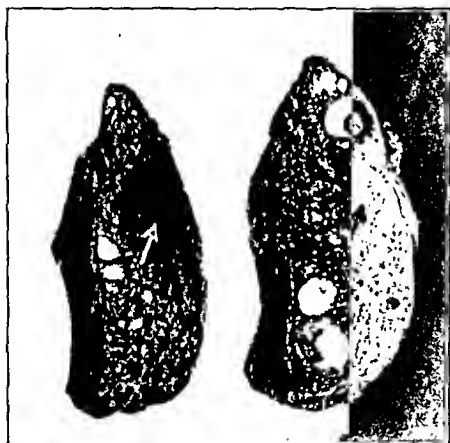
- FIG. 7. Case VI. Photomicrograph of junction of thyroid and chordomatous tissue. The latter stains deeply because of its high mucoid content. The thyroid exhibits advanced fibrosis, lymphocyte accumulation and atrophy of the parenchyma.  $\times 80$ .
- FIG. 8. Case VII. Photomicrograph from periphery of metastasis, showing thyroid tissue penetrated by a large irregular acinus of adenocarcinoma.  $\times 80$ .
- FIG. 9. Case VIII. Vertical sagittal section of the right lobe of the thyroid showing the irregular metastasis with satellite nodules in the caudal parts of the gland (A). (Natural size.)
- FIG. 10. Case VIII. Photomicrograph showing the periphery of the clear-celled renal carcinoma invading thyroid tissue.  $\times 100$ .
- FIG. 11. Case IX. Diffuse invasion of fibrosed thyroid tissue by carcinoma simplex.  $\times 60$ .
- FIG. 12. Case X. Small adenoma of thyroid containing a diffusely infiltrating metastasis denoted by the arrows.  $\times 12$ .



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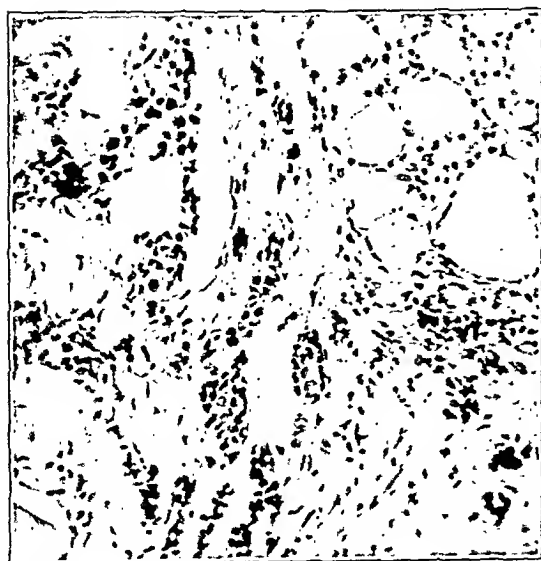
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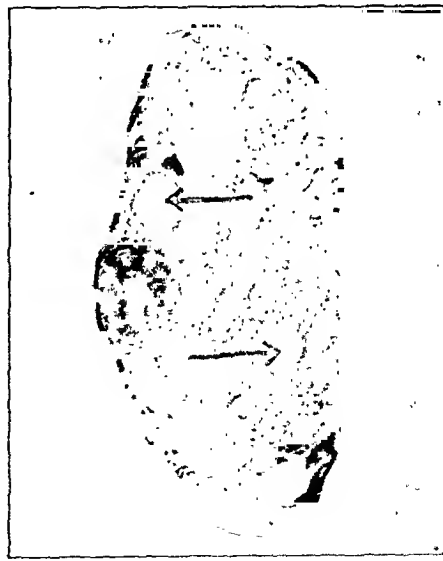
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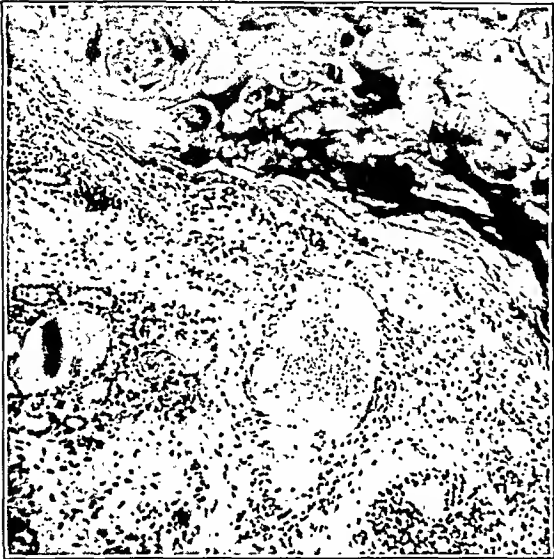


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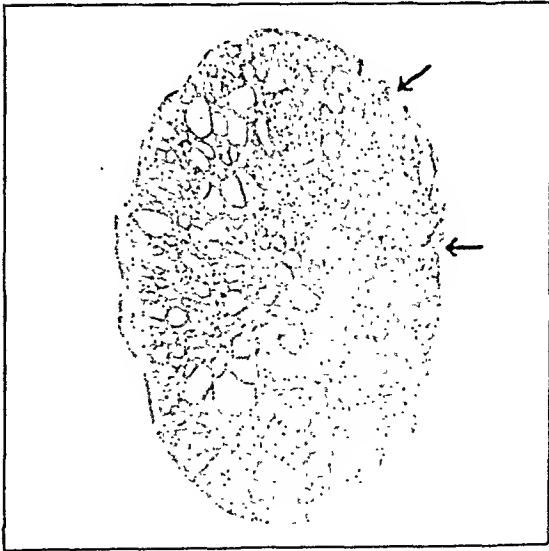
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this technique for the study of tumors. Danchakoff<sup>8</sup> has used the method to grow embryonic chick tissues. Since the publication of these two papers the technique has been used frequently in experiments with auto- and heteroplastic grafts, as well as in those with auto- and heterogeneous tumors. The production of experimental infection in the chorio-allantoic membrane has, however, been done only in the one instance where Rous and Murphy grew the virus of the Rous sarcoma.<sup>7</sup>\*

### TECHNIQUE FOR OBTAINING STERILE VIRUS

One of the most important steps in the technique of virus inoculations of embryonic chick membrane is the use of material free from contaminating microorganisms. The only methods available in the past for obtaining uncontaminated fowl-pox virus have involved considerable dilution of it, such as filtering through a Berkefeld candle or using the virus which capillary attraction has caused to rise higher than bacteria on a piece of filter paper.<sup>9</sup> The virus thus obtained is associated with the minute Borrel bodies in suspension. It was thought that some method which would provide uncontaminated virus consisting largely of inclusions would be especially useful. A number of such methods have been developed recently in connection with this work. Since each may prove advantageous for certain types of experiments, these will be described in detail before continuing with the experiments on embryo inoculation.

METHOD I. Uncontaminated virus can be obtained directly from fowl-pox nodules on the skin of a chick. The following technique proved to be the most satisfactory. After plucking the feathers from the heads of young chicks, 1 to 2 weeks old, virus was inoculated at three points about 1 cm. apart, to allow the development of separate nodules which could be removed by one stroke of the knife. Since nodules of more than seven days' development are likely to be invaded by pyogenic bacteria, the chick was sacrificed six or seven days after inoculation. The head was bathed with 95 per cent alcohol and allowed to dry. With a sterile cataract knife, a nodule was cut off rapidly at a level deep enough to obtain the

\* Mention is made by Askanazy<sup>18</sup> of the production of tuberculous chicks by the infection of fertile eggs.

# THE SUSCEPTIBILITY OF THE CHORIO-ALLANTOIC MEMBRANE OF CHICK EMBRYOS TO INFECTION WITH THE FOWL-POX VIRUS \*

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For studying a representative of the pox group of virus diseases, fowl-pox has many advantages, among them being the fact that infectious material is readily obtained and easily handled, since the disease is limited to fowls. It is believed that knowledge gained concerning this disease may be serviceable in the study of other members of the pox group. Hence, this virus has been the subject of several problems investigated in this laboratory during the last three years.

Fowl-pox, like the other pox diseases, is characterized by the appearance of eruptive skin lesions. The spontaneous fowl-pox nodules appear especially on the unfeathered parts of chickens, although experimental lesions may be easily induced in specialized epidermal structures such as cornea, feather follicles and oil gland. The lesions consist of a hyperplasia of the epithelial cells, with inclusion bodies in their cytoplasm. It has been shown that these inclusions are composed of groups or colonies of minute (Borrel) bodies.<sup>1</sup> While the presence of inclusions has always been considered pathognomonic of the disease, recent experimental work has given much evidence in favor of the theory that the Borrel body, one component of the inclusion, is the etiological agent of the disease.<sup>2, 3, 4</sup>

Heretofore, fowl-pox has been studied only in the grown or newly hatched chicks, or in tissue culture. Tissue culture experiments with this virus have, however, been few and inconclusive.<sup>5, 6</sup> The present paper deals with the inoculation of the virus into embryonic tissues in the incubating egg.

The chorio-allantoic membrane of the chick embryo has been used by a number of investigators for the study of the growth of various implanted tissues. Rous and Murphy<sup>7</sup> were the first to use

\* Received for publication March 7, 1931.

the material into glucose agar, and plating. Proceeding with sterile precautions throughout, it was found that after twenty-four hours in 1 per cent potassium hydroxide, agar plates containing 10 cc. of agar and 1 cc. of a suspension of the treated inclusion bodies usually showed no contamination. Occasionally, however, one or two colonies of a mould persisted. Inclusions freed to this degree from contaminating organisms could then be used for most types of experimental work, though they were less satisfactory than inclusions obtained by the two preceding methods.

METHOD IV. The fourth method for obtaining uncontaminated virus came as a natural development of the successful inoculation of embryonic chick membranes and will be described later. Methods I and IV provide for the production of virus which has never been in contact with bacteria, a fact which should make these virus preparations valuable in immunological experiments.

#### TECHNIQUE FOR INOCULATION OF CHICK EMBRYOS

The technique for opening the eggs used in our experiments was based on that described by Clark.<sup>10</sup> We omitted the use of a hot box, but kept the air sac immersed in water at 39° C. Keeping the air sac thus immersed prevented sagging of the egg contents when the egg was opened. We found that a piece of plasticene molded to fit the egg was a convenient support. The top surface of the egg was sterilized by bathing in alcohol and flaming. Then, proceeding with sterile precautions, a window, 7 to 10 mm. square was made by cutting or scraping with a sharp point. We found the sharp end of a scissors blade very convenient for this. After the shell was removed, the shell membrane was cut away carefully in order to expose the chorio-allantoic membrane.

For purposes of clarity in the description of the inoculation of chick embryos and the chorio-allantoic membrane, a diagram of the 12 day chick with its membranes has been reproduced from Lillie<sup>11</sup> (Text-Fig. 1). One label designating the chorio-allantoic membrane (the membrane formed by the fusion of chorion or serosa with the outer wall of the allantoic sac), has been added to the original diagram.

Two sorts of inoculation were attempted. The simpler procedure consisted in slightly injuring the chorio-allantoic membrane by

infected cores of most of the follicles. The severed nodule was placed epithelial surface down on a sterile glass slide, while, with a pair of fine curved forceps, the infected cores were forced out of the follicles from the cut surface. These small pieces were washed twice with sterile Tyrode's solution and stored at 4° C in a small amount of the same solution. One piece was tested in glucose yeast broth. If no bacterial growth was apparent within twenty-four hours, the remaining virus was made into a suspension for inoculation by grinding with a few drops of Tyrode's solution.

METHOD II. A second method for obtaining uncontaminated inclusions, and one which is especially useful for tissue culture experiments, was developed during work with the inoculation of single inclusions picked out with the Chambers microdissection apparatus.<sup>2</sup> For this work inclusions from lesions of seven to ten days' development were used. The tissue was digested with 1 per cent trypsin to free the inclusions. These were then carefully washed several times with sterile saline. Finally a single inclusion was picked up with a minute sterile pipette and deposited on a sterile cover slip. Though only a few such experiments were tried, plasma cultures with inclusions thus washed remained sterile in every instance. However, unless single inclusions are required, the first method is preferable because it is much less difficult and affords a larger amount of virus.

METHOD III. A third method which is useful when numbers of free inclusions are desired was developed in the course of experiments on the effect of 1 per cent potassium hydroxide on the virus.<sup>4</sup> Since the usual bacteria are destroyed after a few hours in 1 per cent potassium hydroxide, while fowl-pox virus, in the form of inclusion bodies, survives for at least twenty-four hours, we performed a number of experiments to see if virus free from contaminating microorganisms could actually be obtained after one day's treatment with potassium hydroxide. It was found that in highly contaminated pieces of fowl-pox tissue certain moulds, and occasionally a bacillus, persisted for three or more days in 1 per cent potassium hydroxide. After such a long period of treatment the strength of the virus is much diminished and may be completely destroyed. By using inclusions, freed from the tissue by tryptic digestion and carefully washed several times in sterile saline, the number of contaminating organisms was greatly diminished as shown by inoculation of

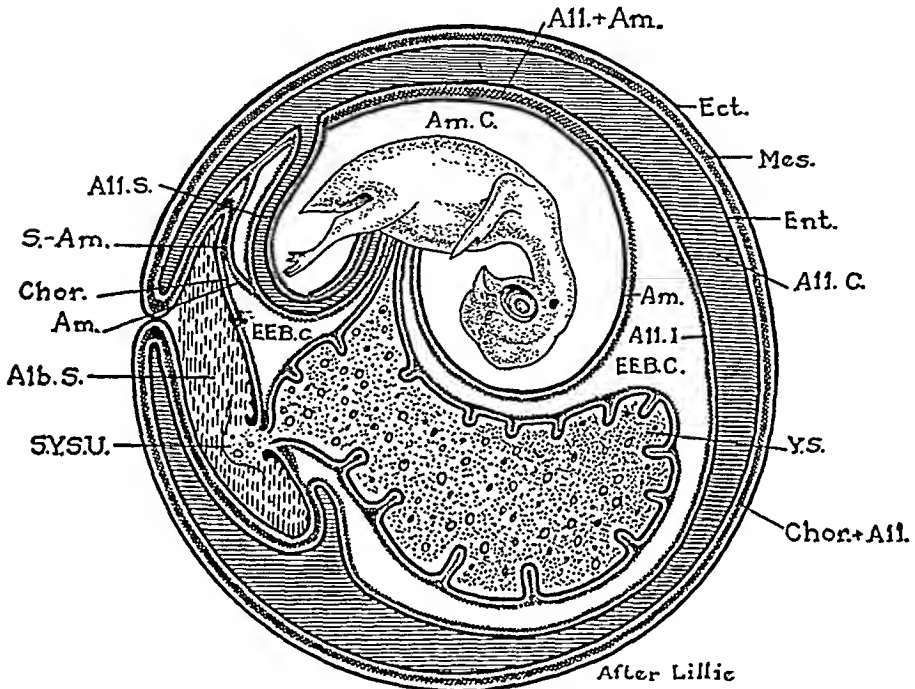
the rest of embryonic growth. The lack of turning caused usually an oval depression and fold in the membrane directly below the opening. No other abnormality due to lack of turning appeared to occur, for a number of chicks hatched normally from eggs which had been subjected to this treatment.

Embryos at various stages of development were used. Since it takes about four days for a well defined fowl-pox lesion to appear after inoculation, it was necessary to inoculate at least that many days before hatching. The most extensive lesions were obtained six to seven days after inoculation, so that 10 to 15 day embryos were used most frequently. Occasionally, a contamination occurred in the inoculation of the egg. Sometimes a mould grew symbiotically with the virus in the embryonic membrane. Such contaminated eggs were discarded. Except as a contaminating organism is introduced upon inoculation, the eggs are relatively free from infection and remain, according to Rettger,<sup>12</sup> a sterile medium, unless subjected to moisture and dirt.

#### RESULTS OF INOCULATION OF EMBRYO CHICK AND MEMBRANES

Fowl-pox infection of the chorio-allantoic membrane occurred as the result of inoculation in every case where the embryo survived for at least four days. Infections were first noted when thickened areas on the chorio-allantoic membrane were detected after several days' incubation. That a fowl-pox infection was definitely present was proved by three tests. The tissue, removed with sterile precautions and inoculated onto the scarified epithelium of adult hens, produced a massive fowl-pox lesion. Smears of the lesions, stained by Morosow's method,<sup>13</sup> showed Borrel bodies present in great numbers. Histological sections of the tissue showed the typical picture of the fowl-pox lesion (Figs. 2 and 9). These lesions are characterized by a marked hyperplasia of the ectodermal layer and an accompanying thickening of mesoderm as well. (Compare with normal membrane (Fig. 1).) Frequently hyperplasia of the entoderm occurs also. In the cells of the ectodermal layer many large inclusions are present, while in the entodermal layer, when occasionally a definite infection is present, inclusions are few and small. The lack of an inflammatory exudate, even in an advanced stage of the infection, should be noted.

pricking with a needle and applying a drop of an uncontaminated virus suspension (Method I) to the injured area. In the second and more difficult operation, the skin of the embryo itself was inoculated. This involved cutting the chorio-allantoic membrane and amnion and slightly abrading the skin of the embryo, since some injury to epithelial cells favors the invasion of the virus.



TEXT-FIG. 1

Alb. S., albumin-sac. All. I., inner wall of the allantois. All. C., cavity of allantois. All. S., stalk of allantois. All. + Am., fusion of allantois and amnion. Chor. + All., fusion of chorion and outer wall of allantois. Am., amnion. Am. C., amniotic cavity. Chor., chorion. Ect., ectoderm. E.E.B.C., extra-embryonic body cavity. Ent., endoderm. Mes., mesoderm. S.-Am., sero-amniotic connection. S.Y.S.U., sac of the yolk-sac umbilicus. Y.S., yolk-sac.

In most of the techniques described in the literature the original piece of shell is replaced following the operative procedure and paraffined, so that the egg can be turned daily to continue normal development. Since we desired to watch the effects of the virus and to get sections at once if the chick should die, we substituted a glass cover slip for the original shell, fixing it upon a ring of vaseline, and returned the egg to the incubator immediately after the operation. This technique necessitated keeping the window uppermost during

Concerning the effect of fowl-pox on embryonic development and ability to hatch, our information is scant since most of the embryos were sacrificed before hatching. A few chicks, however, were hatched from eggs with infected membranes. These chicks were apparently normal, though they must have carried the virus, since all of those that were not sacrificed immediately developed fowl-pox lesions six to eight days later. The nodules appeared most frequently at the base of the beak or about the umbilicus. This infection may have been the result of autoinoculation during the process of pecking through the shell and escaping from the infected membranes. A second possibility is that the cells at the beak and umbilicus, injured during hatching, were infected by virus in the blood stream. The extreme vascularity of the chorio-allantoic membrane would make it seem highly improbable that the blood would remain virus free. Proof of the presence of virus in the circulation was obtained from a series of experiments in which pieces of liver were removed with sterile precautions from chicks which had either hatched from or died in eggs with infected membranes. In the majority of cases where liver material was inoculated onto the scarified epithelium of chicks a small lesion was obtained. In one case out of six, the inoculation of liver material produced no lesion. The lack of massive lesions as a result of these inoculations seemed to us to indicate that though the virus is present in small quantities in the blood stream, it is not actively proliferating there. This observation is in accordance with an accepted view concerning the virus present in the circulation of infected adult hens.<sup>14</sup>

Following the successful inoculation of the membranes of 10 to 15 day embryos, the question arose as to how early in the development of the embryo a successful inoculation could be made. Danchakoff<sup>8</sup> working with embryonic grafts on the allantois states that embryos younger than 7 days could not be used because of the small size of the allantois at that stage. Whether or not the absence of the chorio-allantoic membrane made our inoculations of membranes of embryos younger than 6 days more difficult, it was found that such inoculations were not generally successful. The chorio-allantoic membrane of 6 day embryos was infected with no difficulty, and on one occasion we succeeded in infecting this membrane in an embryo which was inoculated at the 4 day stage (Fig. 8). In younger embryos, the injury caused by opening the egg and pricking the membranes seemed to be greater, for the embryos usually died too soon

In order to show the gross appearance of the infected areas several infected eggs were fixed in Zenker's fluid with the membranes intact. Sometimes the infection occurred in just a few areas, presumably at the site of the original inoculation (Fig. 6), but more often the infected area covered half the surface of the serosa (Fig. 5), and frequently small isolated areas of infection were found at a distance from the large primary lesion.

Upon the discovery that the outer embryonic membrane always developed this large area of infection directly below the window in the egg, it was decided to attempt the removal, with sterile precautions, of pieces of the infected membrane. By flaming the whole egg and carefully removing the coverslip, the infected tissue was exposed. Pieces of the infected membrane were cut away and washed in sterile Tyrode's solution. A sample of the tissue was inoculated into glucose yeast broth, and it was found that uncontaminated material could be obtained readily in most cases. The infected tissue in Tyrode's solution was then stored at 4° C until needed. Virus thus prepared was used generally within two weeks, although samples stored for several months were shown to be still virulent. Uncontaminated virus was obtained by this fourth method in much larger quantities than any other means so far devised. Consequently this method was used in subsequent experiments where such virus was required. The virus obtained by Method IV is convenient for almost any type of fowl-pox problem, if uncontaminated virus is needed, for the material can be used in a number of ways — as bits of infected tissue, as free inclusions which can be teased out from it, or as a Borrel body suspension, made by grinding the tissue with saline.

The inoculation of the embryonic skin caused considerably more trauma than the inoculation of the chorio-allantoic membrane. The percentage mortality was so great that this operation was soon abandoned, since the membrane inoculations proved very satisfactory. Several successful embryo inoculations were made, however. One infection of an embryo foot was produced (Fig. 7), and other infections were obtained, notably at the umbilicus. Although it was not intended to inoculate at this point, injury to the umbilical region must have occurred during the operation, for, with the inoculation of chorio-allantoic membrane alone, umbilical lesions were not obtained except on chicks which hatched and survived for several days.



of the skin of the neck occurred but there were no adhesions to the trachea. In each experiment the mucous membrane of the trachea contained a number of small nodules, sections of which showed the typical fowl-pox lesion (Figs. 12 and 13). The infection of entodermal tissues, both adult and embryonic, is thus shown to be possible under experimental conditions. Uncontaminated virus may prove useful in other experiments of this type. Using such virus, intracerebral inoculations might prove interesting, as well as further inoculations of entodermal derivatives such as the mucous membrane of the intestine, or of mesodermal epithelium, *e.g.*, that in the kidney.

The position of the capillaries in some of the sections of infected chorio-allantoic membrane corroborates Danchakoff's theory concerning the development of the respiratory net of the allantois.<sup>16</sup> The allantois is both the respiratory and excretory organ of the embryo. By the thirteenth to fifteenth day its capillary network has in some manner become the outermost layer of living cells. This is contrary to the usual belief that the mesodermal cells of the embryo must always be bounded by two germ layers. This phenomenon was explained by Füllborn<sup>17</sup> as being due to the degeneration of the epithelial cells of the chorion. Danchakoff, however, holds that the final position of the capillary net is due to a migration of the capillaries. She proved that ectodermal cells were still present, by subjecting them to the pressure of grafted tissue, after which keratinization occurred.

Our sections indicate that migration of capillaries rather than degeneration of epithelial cells has occurred in the change of position of the respiratory net. The ectodermal layer can be distinguished because of the infected epithelium in at least part of the section. If the fowl-pox lesion had developed before the migration of the capillary net, this migration was prevented either wholly or partially. Some sections (Fig. 2) show the capillary net entirely below the ectoderm in the heavily infected area, while it occupies a mid-place in the ectoderm of the same lesion where the infection is less (Fig. 3), and is found on the surface with ectodermal cells below when it reaches a non-infected area (Fig. 4).

In a number of the sections of infected serosa, epithelial pearls were noted, suggesting the possibility that rapid passage of the infection from one embryo to another might result in massive hyper-

after inoculation for a lesion to develop. The technical difficulties involved caused us to abandon an attempt to determine the susceptibility of embryos of less than 4 days' development. It is not intended, therefore, to imply that younger embryos could not be infected.

Detailed study of a number of histological sections of infected chorio-allantoic membrane revealed the fact that entodermal as well as ectodermal epithelium could be infected. Inclusion bodies were usually less numerous, and hyperplasia was less marked than in infected ectoderm (Figs. 9, 10 and 11). Entoderm seems to be much less susceptible than ectoderm since entodermal infection did not occur in every case of successful ectodermal infection. A somewhat similar retarded response of entoderm was found by Huxley and Murray in work with chorio-allantoic grafts.<sup>15</sup> The stimulus in this case, however, was an operative one rather than one caused by an infection.

It should be mentioned in passing that occasionally the membrane was torn at inoculation, and at this point infected ectodermal cells had fused with cells of the entodermal layer. For the identification of a true entodermal infection, however, we were able to obtain sections of isolated nodules at a distance from the point of inoculation, where there was no possibility of ectodermal cells being present in the entodermal layer.

A further indication that entoderm is less susceptible than ectoderm to fowl-pox infection is seen in the fact that entodermal derivatives of the adult hen are rarely infected. Instances of spontaneous lesions in the crop have occasionally been observed. Two instances of spontaneous lesions of the trachea have also been seen. These lesions were not isolated nodules, but part of massive lesions of the throat. Though the infected areas appeared to be in columnar epithelium, the lesions were not considered to be complete proof of the susceptibility of tracheal epithelium (*i.e.* epithelium of entodermal origin), since they were not isolated from epithelium of ectodermal derivation. Using uncontaminated virus (Method IV), an attempt to corroborate the experimental infection of embryonic entoderm by the production of a fowl-pox lesion in adult tracheas was made.

With sterile precautions, the tracheas of two hens were cut, scarified and inoculated. The hens were sacrificed eleven and thirteen days respectively after inoculation. In both cases a gross infection

The experiments recorded in this paper show that the same specificity obtains when this virus is brought into contact with the tissues of chick embryos, and in similar degree. That is to say, in the embryo as in the adult fowl, ectodermal squamous epithelium is more susceptible, while entoderm of the allantois is less readily affected, and in the latter cells, virus regenerates much less abundantly, judging from the number and size of cellular inclusions.

This susceptibility appears very early in the cellular differentiation of the embryo. With the technical methods at our disposal it was not determined how early the embryonic cells acquire this susceptibility, or, to state it differently, how soon certain embryonic cells lose their susceptibility, supposing that the earliest undifferentiated cells from the ovum are all susceptible to the virus. It would be of great interest to know whether or not ectodermal and entodermal epithelium acquire their susceptibility as a result of cellular differentiation.

By the use of the chorio-allantoic membrane of chick embryos for the production of the infection, the preparation of non-contaminated concentrated virus in fairly large quantities is made possible. This virus, being the infected tissue grown in and obtained from a sterile medium, can thus be used in whatever form desired, *i.e.*, as infected tissue, inclusion bodies, or a suspension of Borrel bodies. This method and also Method I, described in this paper, have an advantage over any other known preparations of non-contaminated fowl-pox virus, in that, since the cells in which the virus has developed have never been contaminated, the virus should be free from antigens not directly associated with the disease. This fact should make it especially useful in immunological experiments.

One of the uses of such a virus preparation has been demonstrated in the successful inoculation of adult chicken trachea with fowl-pox virus. Inoculations of such virus into the internal organs of the chicken, especially those with epithelial surfaces such as the kidney, might give valuable information.

The use of embryonic chick membranes as a medium for the production of other virus infections, *e.g.*, vaccinia, might prove advantageous in the study of the etiology and development of these diseases.

plasia resembling an epithelial tumor. Accordingly a series of experiments was begun in which it was attempted to graft bits of infected tissue from the chorio-allantoic membrane on normal membranes. After a period of four to seven days, the original explant plus the newly infected area surrounding it was removed. Part of the block was used for sections and part for transplantation. The explants varied in size from 0.5 to 1.5 c.mm. It was found that identification of the transplant in the gross was difficult, due to its inclusion in the fresh growth of infected tissue. By dipping the transplants in a suspension of India ink in saline, however, enough carbon adhered to the tissue so that the transplant could be identified even after it had produced a heavy infection in the host membrane. Since it was thought that the ink might injure the cells of the transplant, both plain and inked transplants were tried, and several of each type were identified in sections. The series was terminated with the third transplant. Study of the sections obtained showed that in neither inked nor plain transplants had a true graft occurred. Apparently the infected cells degenerated too rapidly for them to become established in the new location. Though hyperplasia was evident, the type of lesion in the second and third transplants was not different from that obtained after the first inoculation. It was concluded that the epithelial pearls were probably due to mechanical displacement of the epithelium in the membrane.

## DISCUSSION

Experiments upon fowls have shown the great susceptibility of ectodermal cells to infection with the virus of fowl-pox. When the virus is injected intravenously the resulting lesions are almost entirely confined to the skin. Sometimes, however, the epithelium of the esophagus, crop and trachea are affected, showing the ability of the virus to multiply in epithelium having an entodermal derivation. From the fact of the infrequent occurrence of spontaneous gastro-intestinal and tracheal lesions, and from the characteristics of these lesions whether spontaneous or experimental, it seems evident that the virus of fowl-pox affects ectodermal epithelium much more readily than entodermal, and increases more abundantly in the former. In common with certain other cytotropic viruses, that of fowl-pox seems thus to possess a high degree of cellular specificity.

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## DESCRIPTION OF PLATES

### PLATE 40

- FIG. 1. Normal chorio-allantoic membrane from hatching chick.  $\times 50$ .
- FIG. 2. Ectoderm of chorio-allantoic membrane of chick embryo showing fowl-pox infection, and the position of the capillary net below a heavily infected area. Section taken five days after inoculation. Note absence of inflammatory exudate.  $\times 200$ .
- FIG. 3. An intermediate position of capillary net in the hyperplastic ectodermal epithelium adjacent to massive fowl-pox infection of embryonic membrane (Fig. 2).  $\times 200$ .
- FIG. 4. Position of capillaries on surface of non-infected ectoderm adjacent to area (Fig. 3) of hyperplastic epithelium in fowl-pox infected membrane.  $\times 200$ .

## SUMMARY

1. Ectodermal and entodermal cells of the chorio-allantoic membrane of the chick, as well as embryonic chick skin, are susceptible to infection with the virus of fowl-pox at an early stage in the development of the embryo. Whether or not this specific susceptibility is acquired as a result of cellular differentiation has not been determined.

2. Four methods for the isolation of uncontaminated fowl-pox virus are described.

3. In two of these methods the virus is developed in tissue that has never been contaminated by extraneous microorganisms.

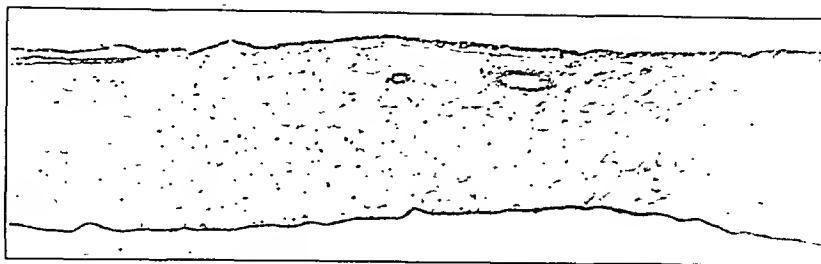
4. Fowl-pox infection in the trachea of the adult hen has been induced by means of inoculation with uncontaminated virus.

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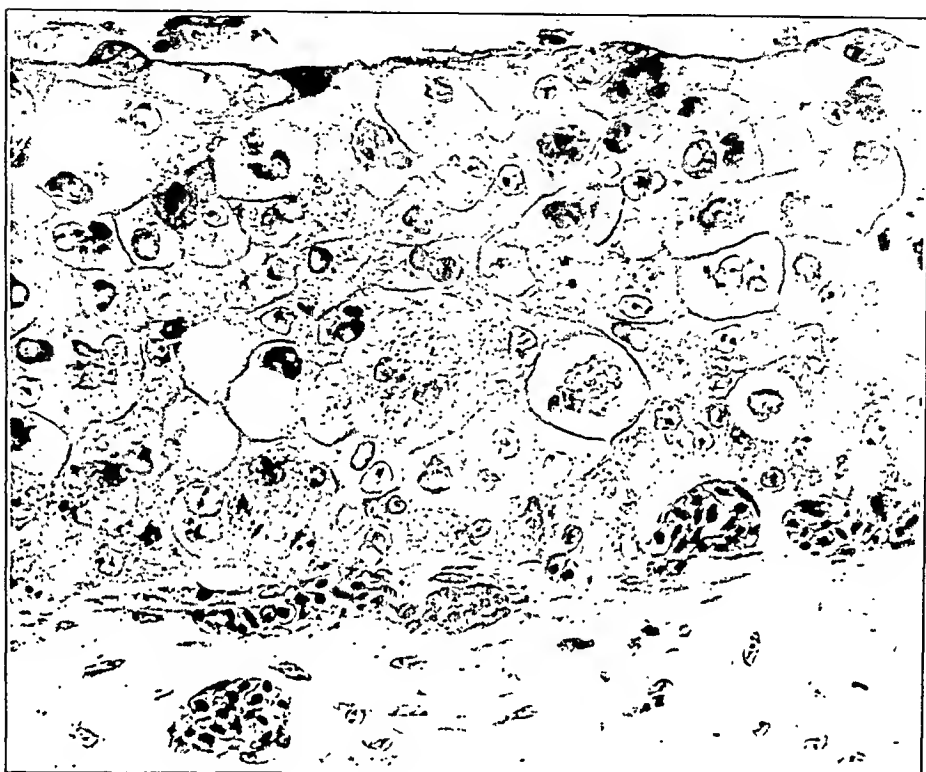
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PLATE 41

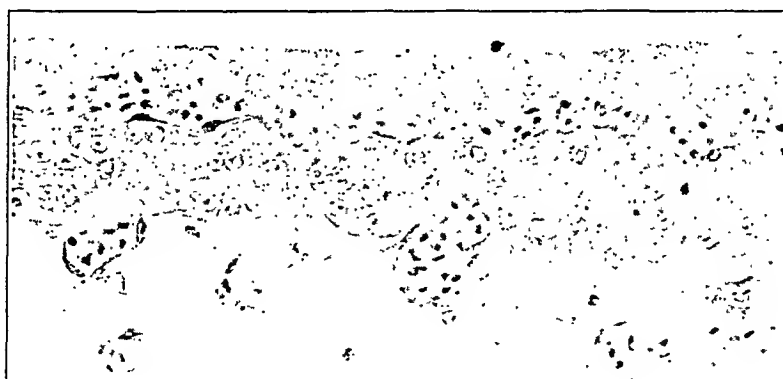
- FIG. 5. Massive fowl-pox infection in chorio-allantoic membrane of 15 day embryo, seven days after inoculation.
- FIG. 6. Isolated areas of fowl-pox infection in chorio-allantoic membrane of 16 day embryo, seven days after inoculation (shown at right).
- FIG. 7. Fowl-pox infection in epithelium of foot of 21 day embryo, seven days after inoculation.  $\times 50$ .
- FIG. 8. Fowl-pox infection in ectoderm of chorio-allantoic membrane resulting from inoculation at 4 day stage.  $\times 50$ .
- FIG. 9. Fowl-pox infection in chorio-allantoic membrane showing massive lesion in ectoderm and hyperplasia of entoderm.  $\times 50$ .



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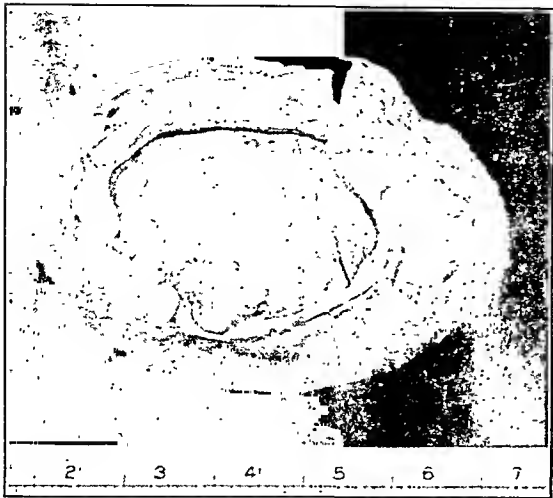


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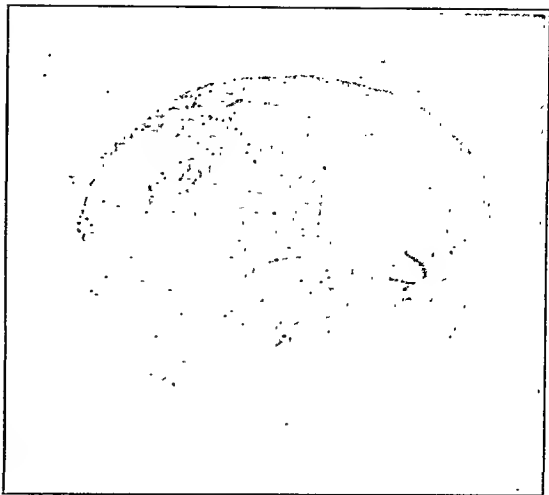


PLATE 42

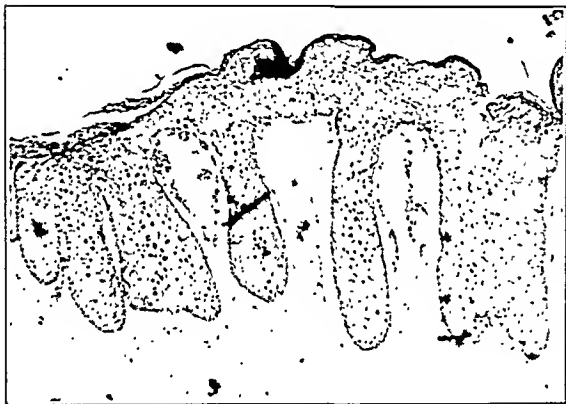
- FIG. 10. Isolated area of fowl-pox infection in entoderm of chorio-allantoic membrane, six days after inoculation. Note small size of inclusions and cells as compared with those of epithelial nodules from ectodermal layer in lower portion of picture.  $\times 50$ .
- FIG. 11. A portion of Fig. 10 under higher magnification, showing fowl-pox infection in entoderm of chorio-allantoic membrane. Note that inclusions are smaller and hyperplasia less than in infected ectoderm of Fig. 2.  $\times 200$ .
- FIG. 12. Experimental fowl-pox infection of mucous membrane of trachea. Cf. normal epithelium at left with infected area at right.  $\times 50$ .
- FIG. 13. A portion of Fig. 12 under higher magnification, showing experimental fowl-pox infection of mucous membrane of trachea. Note metaplasia of epithelium and relatively few inclusions.  $\times 200$ .



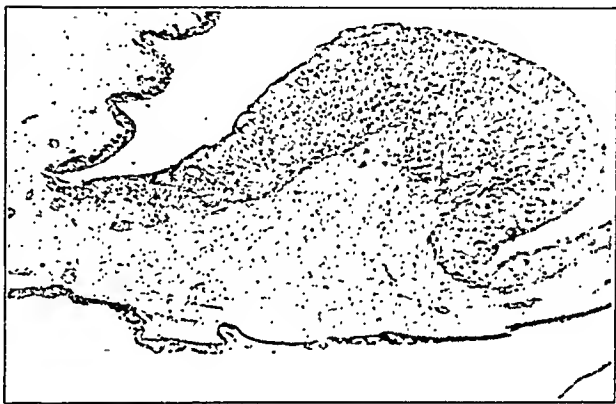
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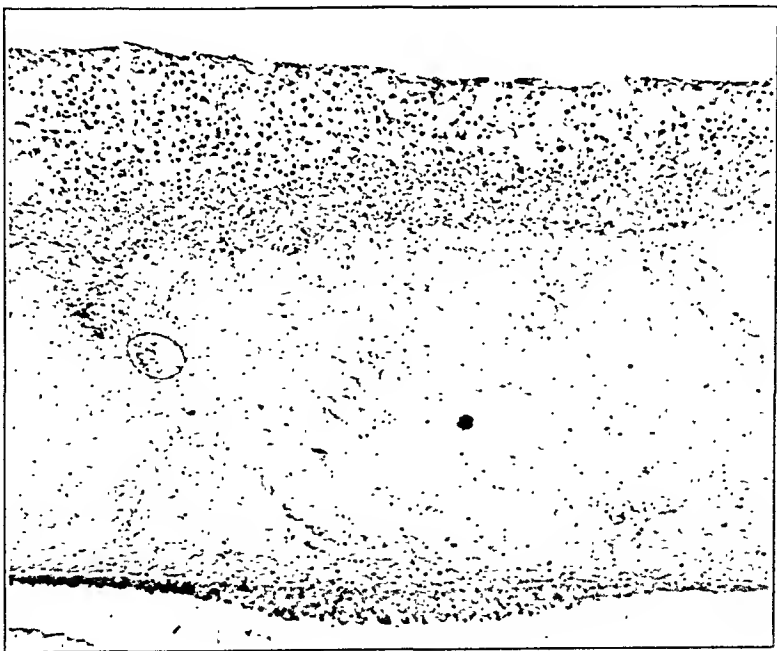
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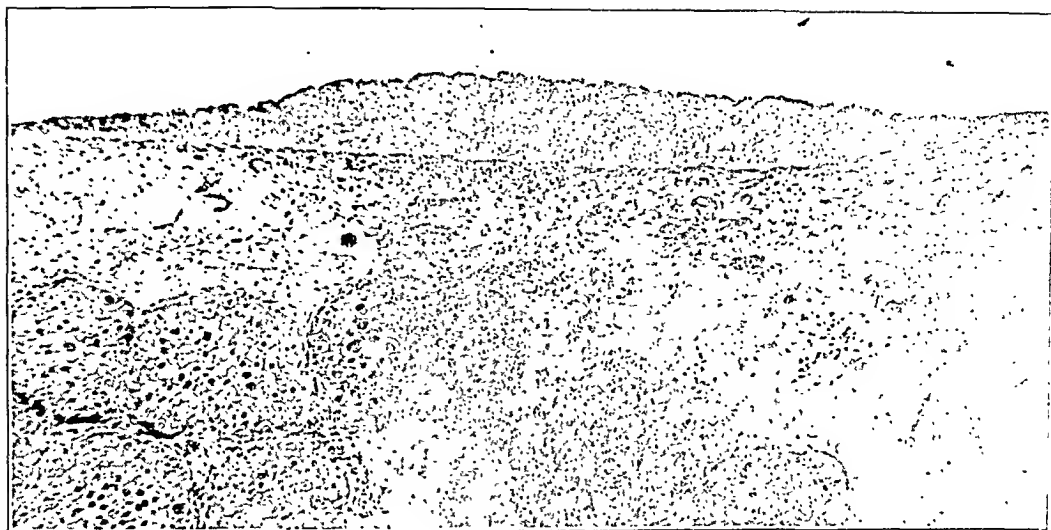


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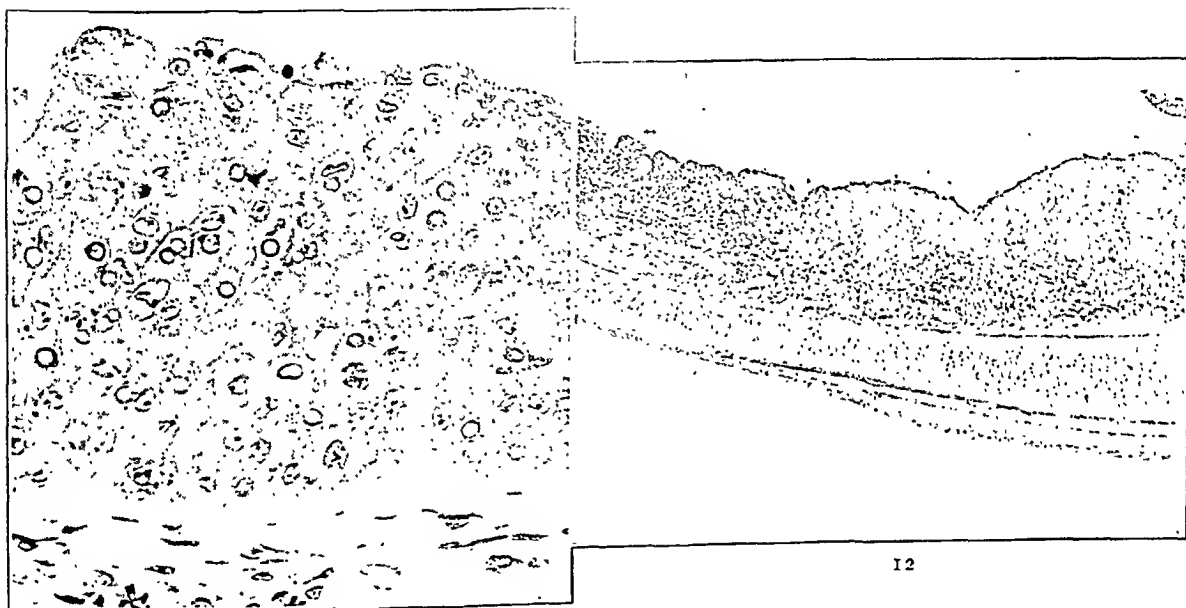


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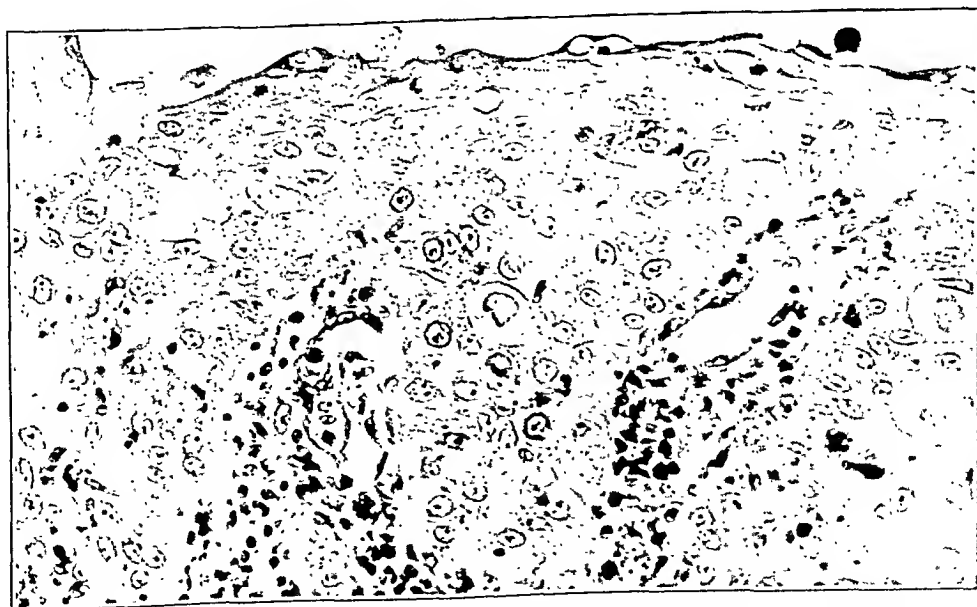




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Susceptibility of Chick Embryos to Fowl-Pox Virus

*Gross Findings at Postmortem Examination:* On opening the thorax the most prominent feature was the ballooned-up pericardial sac which contained more than a liter of blood. The heart showed an excessive amount of subepicardial fat, particularly over the apex, and the surface of the muscle displayed parallel streaks of yellow, alternating with quite normally colored muscle.

On seeking for the source of the blood in the pericardial sac it was found that there was a rupture about 4 cm. in length in the posterior aspect of the left ventricle near the interventricular groove and somewhat tangential to it, the course of the rupture lying roughly parallel, and about half an inch toward the groove, from the small coronary artery on the posterior of the left ventricle (Fig. 1). This artery (D), which largely supplied the area in which the rupture occurred, was somewhat of an anomaly in that the larger branch in this instance came from the right coronary and the smaller anastomosing branch came from the left coronary, which is the reverse of normal.

The rupture showed ragged edges and a gaping slit. The heart was removed and placed in formalin for careful study.

The right auricular appendage contained a thrombus adhering to the lateral wall. The muscle under the thrombus was grayish and translucent. A small branch of the right coronary supplying this area was thrombosed.

In the lateral wall of the right ventricle, just under the tricuspid valve, was another thrombus firmly adherent and of a reddish gray color. Section of the musculature under this thrombus disclosed an area about 3 cm. wide and 5 cm. long, lying just along the margo acutus, which was dark grayish and translucent. The black color of a portion of the center indicated hemorrhage into the area. The artery to this part of the heart was likewise filled with a thrombus. No abnormality of the tricuspid and pulmonary valves was noted.

The left auricular appendage contained no clot and was normal in appearance. The mitral valve showed an irregular calcareous area on the left leaflet, otherwise no pathological changes were noted. The chordae tendineae were normal. The muscle tissue around the rupture was dark, almost black from degenerative changes and altered blood from hemorrhage into the tissues. Adjacent to this darkened muscle there were irregular, grayish, translucent areas throughout a space at least 6 cm. along the interventricular groove

## SPONTANEOUS RUPTURE OF THE HEART \*

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As Beresford and Earl<sup>1</sup> state in a recent report: "The literature on the subject (of spontaneous cardiac rupture) consists mainly of a large number of reports of isolated cases, or very small series, published frequently by quite unskilled observers, struck by the dramatic aspect of the condition." These authors report upon 46 cases of heart rupture among inmates of an insane hospital. Only 14 of the more recent ones had been studied with care histologically, but every one of this number showed recent infarction. Autopsies of the other 32 cases made no mention of infarction, such changes as fatty, friable, soft and degenerated being recorded. This indicates how unreliable opinions as to the cause of the rupture undoubtedly are when made without microscopic examination of the tissues, as many of the 32 cases must have shown infarction which was overlooked because microscopic sections were not prepared.

Krumbhaar and Crowell,<sup>2</sup> and Davenport<sup>3</sup> have reported small series of cases and reviewed the literature, collecting in all over 600 reported cases. They likewise mention the dearth of well studied cases, and bring up several of the unsettled questions in regard to the causes and incidence of rupture. To answer these questions all cases occurring should be studied carefully by the pathologist in an attempt to reconstruct the sequence of events leading to the rupture.

The heart studied in this case was removed by Dr. C. W. Duval at a private autopsy and was kindly turned over to me to be reported.

### REPORT OF CASE

*Clinical History:* A man of 65 years, in apparent good health, with no history of previous disturbances of circulation fell dead while walking upon the street. An autopsy was performed within three hours after death.

\*Received for publication March 2, 1931.

were so advanced that satisfactory sections for photographing were not obtained. All of the larger branches exhibited marked changes such as those seen in Fig. 4. Even the small branches, such as those immediately above the rupture (Fig. 5), displayed such pathological changes as intimal thickening and hyaline degeneration.

*Myocardium:* In sections from an area in the left ventricle near the rupture grossly described as gray and translucent, about midway between the endocardium and epicardium, there was complete degeneration of large groups of muscle fibers, with many fibroblasts growing into such areas (Figs. 6 and 7).

Sections taken at the border of the rupture disclosed a somewhat different pathology. The fibers nearest the rupture had lost their nuclei and had become bright red-staining and homogeneous. A little further away the fibers were likewise without nuclei, but their cytoplasm was coarsely granular. Throughout these areas of hyaline degeneration and cloudy swelling the fibers were widely separated by granular precipitate and leucocytes, principally neutrophiles (Figs. 8 and 9).

The muscle under the thrombi in the right auricle and right ventricle also showed marked changes. Large groups of fibers were hyalinized and stained bright red with eosin. Fibers around these showed pronounced fatty degeneration, while those nearest the endocardium exhibited only granular degeneration. One section from the area in the right ventricle contained large groups of fibers almost completely degenerated, and fibroblasts were beginning to infiltrate between them.

Sections of muscle taken from the left ventricle some distance from the rupture, in what appeared to be normal tissue, did not show very much pathology. The spaces between some of the fibers were widened in places and filled with a fine granular precipitate. There were a few minute clear-cut vacuoles in some of the fibers. Except for this slight edema and fatty degeneration the only other change noted in the myocardium was hypertrophy.

## DISCUSSION

This case bears out the claim made by Beresford and Earl that rupture usually occurs in an infarcted area, frequently the result of coronary disease and precipitated by coronary thrombosis or embolism.

and extending about 4 cm. from the groove into the wall of the left ventricle. The muscle was most changed deep in the wall and appeared more normal toward the endocardium and epicardium. Two large calcareous plaques were found in the aorta just beyond the aortic valves. The valves themselves were normal in appearance.

Interest of course centered in the coronary arteries. The left was rigid and stiff for 4 cm. from the aortic opening. The anterior descending branch was rough and nodular almost to the apex, and on cut section the knife struck hard gritty areas.

The opening of the right coronary artery into the aorta was much constricted, and beginning about 4 mm. from this opening there was an ante mortem clot filling the artery and all of its earlier branches. However, the thrombus did not extend down into the artery supplying the area near the rupture beyond the place where the anastomosing branch from the left coronary joined it, but there was another thrombus filling the distal portion of this artery and its finer branches. In other words, there was no blood or thrombus in this artery between the anastomosing branch of the left coronary and the upper end of the rupture (see portion marked "empty," artery D, Fig. 1).

#### MICROSCOPIC FINDINGS

*Left Coronary Artery:* Sections made at various intervals throughout the course of this artery and its branches showed tremendous thickening of the walls, with advanced degenerative changes. A section taken near the aorta (Fig. 2) is illustrative of the condition of the larger branches. A deep plaque, covering most of the circumference of the vessel, had undergone degeneration to such an extent that no cells were recognizable and various areas contained the slit-like openings left when the cholesterol and other fatty substances were dissolved out; the outer margin of the whole plaque showed considerable infiltration of calcium. The lumen of the vessel was greatly narrowed. Even the smaller branches, such as the terminations of the anterior descending branch (Fig. 3) showed thickening and degenerative changes.

*Right Coronary Artery:* Sections from near the aorta showed the lumen to be very narrow and filled with a thrombus of recent formation. The condition of the walls was quite similar to that of the left coronary, as described above, except that degenerative changes



## DESCRIPTION OF PLATE

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### PLATE 43

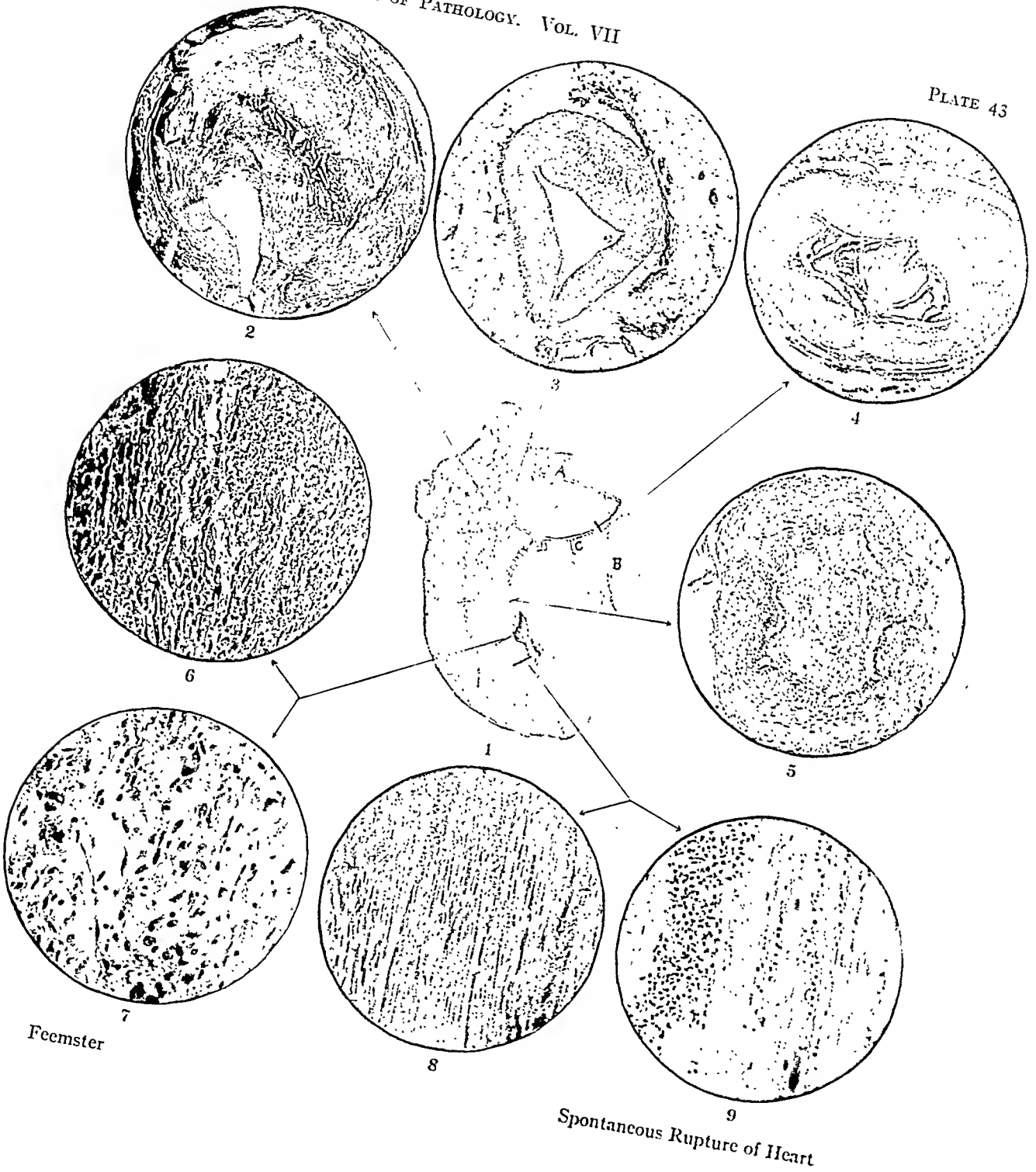
- FIG. 1. Posterior aspect of heart showing rupture in left ventricle.
- FIG. 2. Section of left coronary artery near aorta. Note marked thickening of intima, undergoing degeneration and calcification.
- FIG. 3. Section of smaller branch of same, showing that the arteriosclerosis extends into the terminal branches.
- FIG. 4. Section of right coronary artery about 6 cm. from the aorta. Note the thrombus in the lumen and the degenerative changes in the wall.
- FIG. 5. Section of small branch of same just above the rupture.
- FIG. 6. Section of myocardium near the rupture, showing disappearance of groups of fibers and newly formed connective tissue.
- FIG. 7. Higher magnification of a field of Fig. 6.
- FIG. 8. Section of myocardium at the edge of the rupture, showing various types of degenerative processes and leucocytic infiltration.
- FIG. 9. Higher magnification of a field of Fig. 8.

It is difficult to decide what the sequence of events was in this case, but it appears to have been as follows: The small branches supplying the infarcted areas in the right and left ventricles seem to have been the site of thrombosis or embolism from atheromatous material some days before the rupture, as evidenced by the beginning of repair in the areas supplied by them. It is likely that the vessel supplying the lateral portion of the right auricle became thrombosed shortly afterward. A propagating thrombus then began to form in the right coronary itself (Fig. 1, artery (A)), gradually extending down until it reached the posterior descending branch (C) which runs along the interventricular groove, further cutting off the nutrition to the infarcted area in the left ventricle. When the thrombus finally reached the junction of the terminal branch (D) of the right coronary and the anastomosing branch (E) of the left coronary, nutrition was finally completely cut off from this large area and it became so softened that rupture took place.

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murmur was noted over the base of the heart. The heart seemed to be enlarged, and a thoracic X-ray taken the day after birth revealed an enlarged thymus. The thymus decreased in size under X-ray therapy and the cyanosis gradually subsided. However, during the early months of life great difficulty in feeding was experienced. Within two months after birth the child was recognized as a Mongolian idiot, the characteristic stigmata of this condition gradually developing.

Starting at the age of 6 months with an attack of bronchitis, the patient suffered from recurring pulmonary infections associated with severe cyanosis. Between these attacks cyanosis was absent. Death finally occurred in the course of a mild attack of scarlet fever, the patient, while seemingly in little danger, suddenly became extremely cyanotic and died within a few hours.

The cardiac condition was repeatedly studied during the life of the patient. The heart was definitely enlarged, especially to the right, so that it seemed more centrally located than usual. Over the base there was a loud blowing systolic murmur that was not transmitted to the great vessels. During the attacks of cyanosis which accompanied respiratory infections, this murmur increased in magnitude. Exertion never was a factor of importance because the child was inactive. At the age of 2 years she learned to stand, while holding to something, but never was able to walk independently.

### DESCRIPTION OF HEART

The heart, while normal in shape, is greatly hypertrophied, weighing 183 gm. (normal for age about 80 gm.),<sup>4</sup> and shows a slight dilatation of all chambers. The right ventricular wall approximates the left in thickness, the right measuring 13 mm. and the left 14 mm. in width. Both auricular walls are moderately thickened. The interauricular septum is complete above and shows a normal fossa ovalis (Fig. 2). The septum is incomplete below, its crescentic free border arching over a large common auriculoventricular orifice to join its anterior and posterior margins at the base of the auricles, thus forming a free communication between the two auricles (Fig. 2). This opening measures 1.6 cm. in anteroposterior diameter and 0.6 cm. in height. Above, it is bounded by the arching free border of the interauricular septum. Below, it is separated partially from the large defect of the interventricular septum by the incomplete diaphragm formed by the central segments of the common auriculoventricular valve. The free margin of the interventricular septum arches downward from the anterior and posterior margins of the auriculoventricular orifice to form an opening through which the two ventricles directly communicate (Figs. 3 and 4).

There is a single large auriculoventricular orifice which is slightly constricted in the middle (Fig. 1). The right half of this functions

## CONGENITAL HEART DISEASE \*

### A PERSISTENT OSTIUM ATRIOVENTRICULARE COMMUNE WITH SEPTAL DEFECTS IN A MONGOLIAN IDIOT

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A defect of the adjoining portions of the interauricular and interventricular septa with a common auriculoventricular orifice is an unusual cardiac anomaly. In 1927 Gunn and Dieckmann<sup>1</sup> reported two such cases, both occurring in Mongolian idiots. They were able to find six cases previously described in the literature and quote Keith as stating that he had seen fourteen and as citing two more, making a total of twenty-two such cases which they found described or referred to in the literature prior to their report. However, Keith<sup>2</sup> did not describe his cases, but simply stated that he had seen fourteen hearts with common auriculoventricular orifices and that this condition was always associated with other grave defects, such as pulmonary stenosis or transposition of the great vessels. Neither of Gunn and Dieckmann's cases had any associated major defect (one had a patent foramen ovale and a patent ductus arteriosus), and of the six cases which they found described in the literature only two showed associated defects, namely a bicuspid pulmonic valve in one and a partial transposition of the aorta in another. Mönckeberg<sup>3</sup> also described a case, not included in those found by Gunn and Dieckmann, which showed no associated defect other than a small patent foramen ovale. From this it is evident that a common auriculoventricular orifice and septal defects, uncomplicated by other grave anomalies, is of rare occurrence.

#### REPORT OF CASE

*Clinical History:* The patient was a white female who died of scarlet fever at the age of 4 years and 9 months. The three older children of the family were normal. This child was extremely cyanotic at birth, although it was a full-term baby with a normal labor and easy low forceps delivery. At birth a loud systolic

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depression in the systemic aorta at the site of its attachment. The pulmonary artery and its branches are very large. The systemic aorta is 4 cm. in circumference, while the pulmonary artery measures 6.5 cm. Its branches are also large, the left 3 cm. and the right 4.5 cm. in circumference. The pulmonary artery shows several very small yellowish patches of atherosclerosis. None are present in the systemic aorta. The cavae, coronary sinus, and pulmonary veins are all normal and enter the auricles in the usual manner. The Eustachian valve is well formed.

Other positive findings at autopsy were a terminal bronchopneumonia and an interesting anomalous condition of the ovaries. Two small bodies, each about 6 mm. in diameter, were found attached to the posterior surfaces of the broad ligaments in the position normally occupied by the ovaries. Except for their small size they appeared to be normal ovaries. However, on microscopic examination they were found to be composed of a vascular and fibrous stroma containing a large number of tubular structures lined by high columnar epithelium, evidently vestigial wolffian tubules. A careful search of serial sections through each of these bodies revealed a complete absence of any ovarian follicles or germinal epithelium.

## DISCUSSION

The interpretation of this type of cardiac anomaly is extremely interesting, but is complicated by the uncertainty regarding the exact process by which the final complete separation of the two sides of the heart is accomplished normally. Particularly is this true in regard to the relative importance of the parts played by the septa and the endocardial cushions. Mönckeberg <sup>5</sup> believes that the septa play the larger part, the endocardial cushions growing out along the margins of the interauricular and interventricular septa when the septa reach the level of the auricular canal. He states that the presence of the margin of one or the other of these septa at the level of the auricular canal is a prerequisite for the fusion of the endocardial cushions, and that the absence of the margins of both septa at this level necessitates the persistence of the primitive single auriculoventricular orifice. From this it would follow that the primary fault in these cases is a growth deficiency in both the interauricular and the interventricular septa. Thus the common auriculo-

as the right, and the left half as the left auriculoventricular orifice. This large orifice is guarded by a valve composed of five segments. There are two large mesial segments, one anterior and the other posterior, each lying half in the right and half in the left heart. The right halves of these two segments represent the septal segment of the tricuspid valve, while the left halves correspond to the aortic segment of the mitral valve. On the right there are two additional divisions — the normal anterior and posterior tricuspid segments. On the left a normal posterior mitral segment is present. The arrangement of the valve segments is illustrated in Fig. 1. The large posterior central segment is attached closely to the margin of the underlying interventricular septum by a group of partially fused, short, cord-like strands of connective tissue; thus there is very little communication between the ventricles in this location. Under the anterior central segment the defect of the interventricular septum is quite deep, this segment being attached to the margin of the defect by only one large branched chorda tendinea (Fig. 4).

The defect of the interventricular septum is continuous with the auricular defect, the two being separated only by the incomplete diaphragm formed by the large central segments of the auriculoventricular valve. Its anteroposterior diameter is the same as that of the defect of the interauricular septum (1.6 cm.). Its greatest depth is 0.6 cm. and this lies under the anterior central valve segment. The defect extends well forward under the aortic orifice on the left (Fig. 4). The opening into the right ventricle lies under the central valve segments, especially under the anterior one and behind the conus, which is quite thick-walled (Fig. 3). The endocardium in this region is thickened and hyalinized. Several thickened chordae tendineae arise from this site, some of which pass into the left heart to be attached to the valve there. An especially large branching chorda arises from the free margin of the defect (Fig. 4). The remaining chordae are normal.

The orifice of the systemic aorta, which lies immediately above the anterior portion of the defect in the interventricular septum, is guarded by three normal cusps. It measures 4.5 cm. in circumference. The pulmonic orifice measures 5 cm. in circumference and also has three normal cusps. The systemic and pulmonic aortae are free from anomalies. The ductus arteriosus is not patent. It is represented by a fibrous cord connecting the two with a dimple-like



Abbott <sup>8</sup> seems to fill partly the gap between the cases of persistent ostium primum with deformed auriculoventricular valve segments, normal auriculoventricular orifices and intact interventricular septum, and the type of case we have reported. In Abbott's case the aortic segment of the mitral valve was not only cleft, but was completely divided and the upper part of the interventricular septum "appeared to be slightly defective below." As Gunn and Dieckmann point out, while embryologists are not agreed as to the exact mechanisms of the process, it is safe to assert that the final closure of the interventricular foramen is brought about by the fusion and growth of three structures, namely the bulbar septum, the interventricular septum, and the endocardial cushions. A deficiency in any one of these three could lead, therefore, to a defect in the base of the interventricular septum. We believe Abbott's case, mentioned above, to represent a slight defect of the interventricular wall brought about by a failure of downward growth of the endocardial cushions, and our case to represent a more severe defect originating in a similar manner.

To summarize this discussion, it is suggested that a persistent ostium primum with deformed valve segments is due to a failure on the part of the endocardial cushions to grow up and unite with the interauricular septum, and not to a failure of the downward growth of that septum. In these cases fusion of the endocardial cushions occurs in the auricular canal, but even there it is not complete or normal, as shown by the cleft valve segments. If the endocardial cushions are further arrested in their growth the segments are not only cleft, but completely divided with a smaller (Abbott's cases) or larger (our case) defect of the base of the interventricular septum. The plausibility of this explanation is strongly supported by Mall's observations and it has the practical advantage of explaining the defects observed in our case on the basis of a single primary growth deficiency of one structure (the endocardial cushions) rather than by the coincidental failure of two or more structures. However, it should be admitted in passing that defects of the base of the interventricular septum do occur without the slightest evidence of faulty development of the endocardial cushions. Since, as stated above, the processes of fusion and growth of the bulbar and interventricular septa take part in the closure of the interventricular foramen, these defects, as is generally accepted, are due to a deficiency in the septum

ventricular orifice is not due to any defective development inherent in the endocardial cushions, but results from the absence of a septal margin at the level of the auricular canal. On the other hand, Gunn and Dieckmann, following Mall, believe that the final closure of the ostium primum (primary interauricular foramen) and probably of the interventricular foramen is brought about by the fusion and growth of the endocardial cushions. They conclude, therefore, that the primary fault is a growth deficiency on the part of these structures. For a detailed discussion of this problem the reader is referred to the article by Gunn and Dieckmann.<sup>1</sup>

The author is inclined to believe that their position is the correct one. The careful work of Mall,<sup>6</sup> which is stressed by these authors, is especially convincing. Mall observed in a human embryo of 8 mm. the upward growth of the anterior and posterior endocardial cushions encroaching upon the ostium primum and uniting with the interauricular septum well above the auricular canal. This condition is clearly shown in Mall's illustrations. In an embryo of 9 mm. he found the endocardial cushions fused within the auricular canal, while the interventricular foramen was still open. In the case of this embryo he does not state whether or not the ostium primum was closed. This is strong evidence that the endocardial cushions play a very large part in the closure of the ostium primum and that they do fuse before the interauricular and interventricular septa reach the level of the auricular canal. In support of the opinion that a primary deficiency of growth on the part of the endocardial cushions is responsible, at least for the valvular anomaly and the defect in the lower part of the interauricular septum, is the fact that in cases of persistent ostium primum, uncomplicated by a defect at the base of the interventricular septum, there is commonly an associated anomaly of the valve segments, the aortic leaflet of the mitral valve being cleft from its free border to its insertion (Abbott 7). This suggests that in such cases the fault may be in the endocardial cushions rather than in the development of the interauricular septum.

Gunn and Dieckmann also discuss in detail the defect of the base of the interventricular septum, concluding that there is little evidence in their cases that the interventricular septum was deficient; therefore the defect was due probably to a failure of downward growth of the endocardial cushions. Their reasoning applies with equal force to our case. In this connection, a case reported by

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## DESCRIPTION OF PLATES

### PLATE 44

FIG. 1. The auricles have been partly cut away and the defective interauricular septum reflected exposing the common auriculoventricular orifice with its five valve segments. The two large mesial segments are clearly seen, the anterior above and the posterior below. The right halves of these two segments represent the septal segment of the tricuspid valve, the left halves the aortic segment of the mitral valve. To the right two small segments represent the anterior and posterior tricuspid segments. To the left a single posterior segment is present, the normal posterior mitral segment.

FIG. 2. The right auricle and ventricle are laid open. A large defect of the lower portion of the interauricular septum (persistent ostium primum) is present with the free margin of the septum arching over it. Below this defect the right halves of the mesial segments of the valve guarding the common auriculoventricular orifice are seen (*cf.* Fig. 1). Between these segments there is a deep notch representing the defect in the base of the interventricular septum. The fossa ovale is present in the upper portion of the interauricular septum. The wall of the right ventricle is greatly hypertrophied.

and belong in a different category from the defect associated with a persistent ostium primum and a common auriculoventricular orifice.

The frequency of congenital heart disease in Mongolian idiots is well recognized, Cassel<sup>9</sup> finding it in eight of sixty cases and von Hofe<sup>10</sup> in fourteen of one hundred and fifty cases. Abbott,<sup>8</sup> reporting the case of persistent ostium primum referred to above, emphasizes this fact and states that in her experience the cardiac defect not infrequently is a persistent ostium primum. Of the nine cases of persistent ostium atrioventriculare commune with septal defects, which we have found in the literature, four have occurred in Mongolian idiots — both of Gunn and Dieckmann's cases,<sup>1</sup> one of the six which they cited from the literature, and the one reported by Mönckeberg.<sup>3</sup> With our cases added, five of the ten reported cases have occurred in Mongolian idiots.

### SUMMARY

1. A case is reported in which a persistent ostium atrioventriculare commune is associated with a defect in the base of the interventricular septum and a persistent ostium primum. This occurred in the heart of a Mongolian idiot who showed also complete absence of true ovarian tissue.

2. The cardiac defect is believed to be due to faulty development of the endocardial cushions.

3. Four of the nine similar cases found reported in the literature occurred in Mongolian idiots.

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PLATE 45

FIG. 3. The right auricle and ventricle are laid open. The defect of the lower portion of interauricular septum and of the base of the interventricular septum are shown with the anterior mesial segment of the auriculoventricular valve extending through this opening (the left-hand portion in shadow). The endocardium of the interventricular septum near the anterior margin of the defect and of the posterior wall of the conus is much thickened, having a white hyaline appearance. From this region spring a number of chordae tendineae which are attached to the anterior mesial segment and to the anterior tricuspid segment (*cf.* Fig. 1). Some of these chordae are seen extending into the left heart through the defect. Just below the area of thickened endocardium the roomy, thick-walled conus is seen.

FIG. 4. The aorta and left ventricle have been laid open. The aorta is normal. Below its orifice the defect of the base of the interventricular septum is seen. From the free margin of the septal defect arises one large branched chorda tendinea which is attached to the anterior mesial segment (*cf.* Fig. 1).

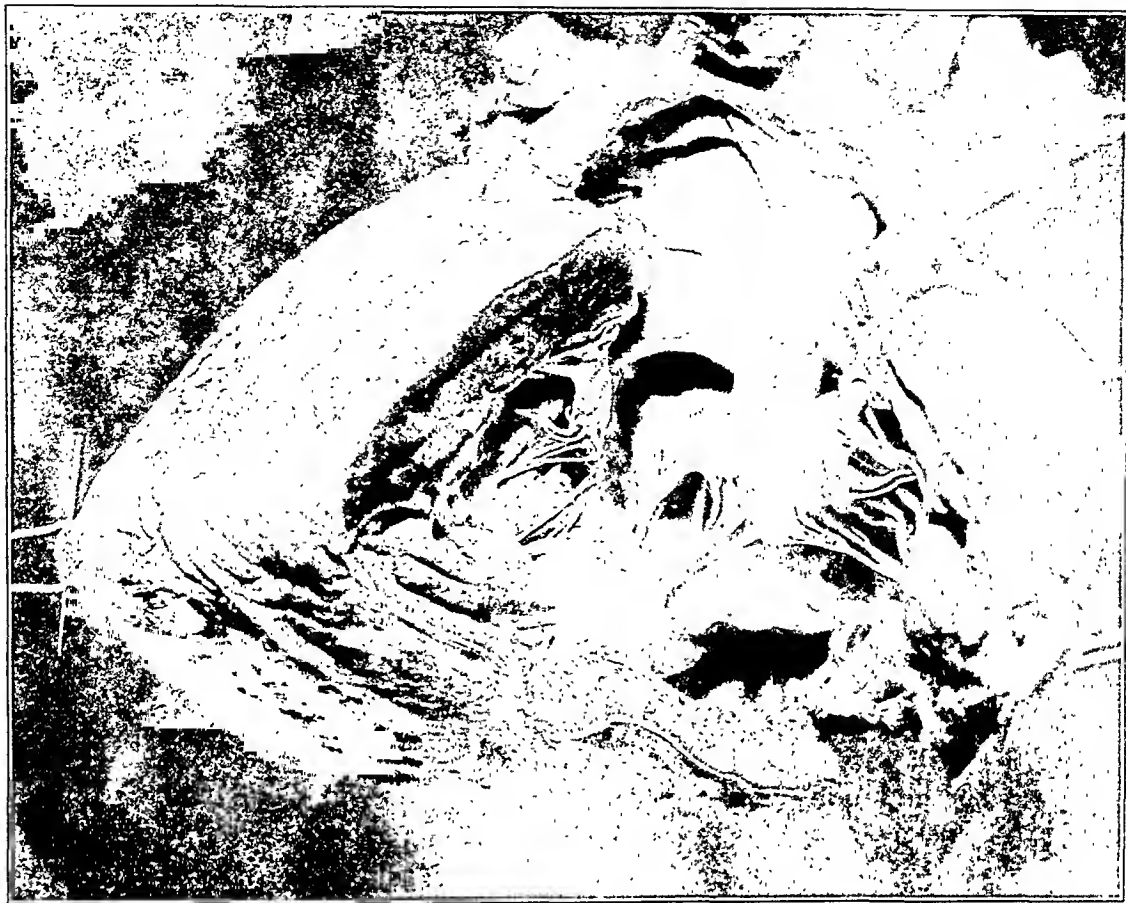


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handling the advertising gave what purported to be a list of ingredients of Weldona, although no quantities were given. The list was: Neocinchophen, Extract of Cimicifuga, Fluid Extract of Phytolacca, Magnesium Carbonate, Light and Powdered Extract of Cascara Sagrada." "A commercial laboratory that analyzed Weldona in September, 1927 . . . did not satisfactorily prove the presence or absence of neocinchophen, but did report that tests for alkaloids showed none present." In Cabot's case the liver appeared to be severely involved in an atrophic process which was recognized as acute atrophy or acute hepatitis. There was associated ascites, with collection of 2500 cc. of fluid, and esophageal varices were also found. Cases similar to these have now been reported by several investigators. Thus Rabinowitz,<sup>8</sup> in 1930 was able to review fifty cases with twenty-five deaths, twenty of which were followed by autopsy. Since Rabinowitz' review, Parsons and Harding<sup>9</sup> have published complete protocols of four fatal cases. The evidence at autopsy in every case thus far reported points to the liver as being the principal site of pathological change, so that the fatal toxic manifestations from cinchophen, in the liver, seem established beyond coincidental relationship.

Five cases of fatal toxicity from the use of preparations of cinchophen have been seen at The Mayo Clinic. These cases lend added confirmation to those previously reported, indicating that the toxic manifestation of cinchophen is principally exerted on the liver, that the lesion produced is essentially destruction of hepatic parenchyma, and that the process is pathogenetically related to toxic cirrhosis. In these cases the preparations of cinchophen had been either self-administered or taken without adequate medical supervision. The patients came to The Mayo Clinic to seek relief from jaundice and related physical disturbances.

### NATURE OF THE TOXIN

Certain persons possess apparent immunity to the use of cinchophen and others are clearly hypersusceptible. Hench and Rowntree<sup>10</sup> saw a patient who had taken cinchophen in large amounts over a period of eighteen years without the slightest discomfort or disability. One of Reichle's<sup>11</sup> patients had taken 458 gm. of the drug over a period of three years before showing definite evidence

# THE SPECIFIC CHARACTER OF TOXIC CIRRHOSIS AS OBSERVED IN CINCHOPHEN POISONING \*

## A REVIEW OF FIVE FATAL CASES

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Cinchophen (phenylquinolincarboxylic acid) was introduced to medicine in 1908 by Nicolaier and Dohrn,<sup>1</sup> and marketed under the trade name of "atophan" (tophi remover). It was originally intended as an eliminant of uric acid in the treatment of gout. Subsequently, because of the similarity of its action to that of the salicylates, it was widely recommended for relief of pain in the various rheumatoid affections. It has formed the basis of many of the well known "rheumatism cures," some of which are so labelled that the name gives no indication that they contain derivatives of cinchophen. Unfortunately, knowledge of its toxic properties did not appear until its use had become general.

Von Müller,<sup>2</sup> Phillips,<sup>3</sup> and Herrick,<sup>4</sup> in 1913 reported that in the course of administration of cinchophen, signs of toxicity became manifest in certain persons. They described the reaction as an urticarial or scarlatiniform rash. Schroeder<sup>5</sup> in 1922, although emphasizing the therapeutic value of the drug in the treatment of gout, indicated its liability to induce toxic manifestations. His cases were illustrative of rather mild poisoning and presented symptoms of headache, gastro-intestinal disturbances and transient jaundice. Worster-Drought<sup>6</sup> in 1923 reported a case of more severe intoxication, but with recovery, and stated that in addition to the symptoms mentioned by Schroeder, jaundice should definitely be added. In 1925 R. C. Cabot<sup>7</sup> reported the death, from acute yellow atrophy of the liver, of a patient who had taken "Weldona" tablets. Concerning "Weldona," the *Journal of the American Medical Association*, for October 1, 1927 contained the following: "the advertising agency

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a period of five months, in four courses, each time with early developing evidences of toxicity, such as pruritus and anorexia, until finally, persistence in its use induced grave icterus from which the patient died after four weeks of illness. In the other case (Case 5) of toxicity from oxyliodide a similar story was elicited. This patient rather unwillingly admitted the use of the drug, but evidence was adduced indicating its use for about two weeks. At the end of that time jaundice had appeared. When he ceased to use the drug he improved, only subsequently to resort to its use; toxicity returned and death resulted about five months following the first toxic manifestation.

Some have thought that the variable toxicity is a manifestation of impurity of the drug. Willcox suggested that the toxic principle was the quinolin nucleus, common to all preparations of cinchophen. He advanced his argument by analogy of arsenical compounds; the preparations of arseno-benzol have much greater toxicity for the liver than either inorganic or aliphatic compounds of arsenic. The increased toxicity of the arseno-benzol group, he thought, was due to the benzene nucleus. Since the quinoline nucleus consists of a benzene and a pyridine ring which can yield the toxic ring of free benzene, he suggested this derivative as the actual toxic principle. Sutton<sup>23</sup> advanced the hypothesis that the toxicity was due to the oxidation of the quinoline nucleus into highly toxic nitrocompounds; this would explain the variability in toxicity by the proportionate oxidative disintegration of the original preparation. Rabinowitz took cognizance of the facts that have been given here, but in addition emphasized that the predisposition may rest in the constitution of the patient, as influenced by previous hepatic disease, malnutrition, or any condition which favors decreased storage of glycogen in the liver. From experimental evidence adduced from the use of other toxins which specifically affect the liver, Opie and Alford,<sup>24</sup> Simonds,<sup>25</sup> Graham,<sup>26</sup> Davis and Whipple,<sup>27</sup> and others have indicated that, to some extent, glycogen affords definite protection to the liver, both in its ability to offset toxic destruction of hepatic cells and in its ability to influence the regenerative capacity of the liver following injury.

of toxicity. On the other hand, Kessel's<sup>12</sup> patient showed toxic effect with 4.5 gm., and Evans'<sup>13</sup> patient required only about 1 gm. before toxicity became apparent. Worster-Drought's patient had taken 270 grains (18 gm.) in twelve days when an urticarial rash appeared. Taking of the drug was discontinued. After an interval of three weeks it was thought safe to resume the medication; after the first dose, of 7.5 grains (0.48 gm.), more severe toxic manifestations with jaundice appeared. In most instances signs of toxicity have appeared only after use of a considerable quantity of the drug over a relatively long period, or they have become manifest after treatment has been completed, as in the fatal case reported by Willcox,<sup>14</sup> and in one of Loewenthal, Mackay and Lowe's<sup>15</sup> cases in which jaundice appeared two weeks after medication had been stopped. Immediate reactions have been recorded with minimal amounts of the drug following its intravenous use, as in the cases of Kingreen,<sup>16</sup> Hitzenberger,<sup>17</sup> Schwarz,<sup>18</sup> Singer,<sup>19</sup> and Haudek<sup>20</sup> in which diiodo-atophan (biloptin), because of its high content of iodine and its excretion through the liver, was administered in the course of diagnostic cholecystography. Most of the fatalities have developed when administration of the drug was without proper medical supervision, but Willcox and Loewenthal exercised every known precaution in their cases, in spite of which toxicity was encountered.

In cases seen at The Mayo Clinic, because of the manner in which the drug was taken, exact knowledge of the amount used could not be obtained. In one case previously reported by McVicar and Weir<sup>21</sup> (our Case 4), the patient had taken an undetermined amount of atophan for about one year before definite signs of toxicity appeared. In another case reported by Stacy and Vanzant<sup>22</sup> (our Case 2), the patient had taken from one to three tablets of cinchophen for three weeks before realizing that the drug was toxic for her. In another case (Case 1), not reported previously, the patient had taken about twenty-five tablets of cinchophen for only five weeks, according to his story, when a fulminating illness appeared which ended fatally after twelve days of jaundice. Evidently a relatively small amount of drug was taken over a short period of time, yet it was the most fulminating of any of our cases. In two other cases hitherto not reported, the cinchophen was used in the form of oxyliodide. In one of these (Case 3), the patient had taken the drug over

hepatic cords with rounded terminal ends pointing toward the central vein. These structures were surrounded by collapsed sinusoids and their supporting reticulum. Some of these tubular structures were branched, of irregular size and shape, and appeared to blend in some instances with formation which closely resembled biliary ducts, but which still lay in the intralobular zones. Transitional stages existed from field to field, indicating that the pseudotubuli were remains of disassociated hepatic cords. In support of the morphological evidence of the hepatic derivation of these tubules was the fact that the cells contained lipoids. Interlobular biliary ducts did not contain fat. Only a slight amount of fat was present in the disorganized hepatic cords, except in those in a peripheral situation such as the tubules just described. Further evidence of their hepatic function was shown by the occasional presence of biliary thrombi in them. Proliferation of interlobular biliary ducts was not seen. They were more prominent because of the destruction of surrounding hepatic parenchyma. At the borders of the interlobular zones some tubular structures were observed which had the appearance of biliary ducts, in addition to the previously described tubules. These appeared to represent the terminations of intralobular biliary ducts. Little evidence of regeneration of hepatic cells existed, recovery from the initial injury apparently not having been completely made, but a few clusters of apparently newly formed hepatic cords appeared, always lying near the interlobular structures. These were exhibited as widened hepatic cords containing enlarged hepatic cells with narrow sinusoids between the cords; thus an apparent compensatory hypertrophy-hyperplasia of well preserved, persisting hepatic units resulted. Mitotic figures were rarely found. Leukocytic infiltration was sparse; a few cells of mononuclear and polymorphonuclear types appeared to invade the interlobular connective tissue and bare sinusoidal portions. Connective tissue did not anywhere appear to be newly formed.

*Comment:* The diagnosis was atrophy of the liver induced by cinchophen, an early stage of toxic cirrhosis. The hepatic changes appeared to be further advanced than the history would indicate. This appearance may be deceptive; one inclines to the view that the changes are far advanced on account of the predominance of connective tissue; however, its presence may be correctly interpreted as only the survival of stromal and vascular structures uninvolved

## REVIEW OF FIVE FATAL CASES

CASE 1. A man, aged 37 years, had taken cinchophen (about twenty-five tablets) for arthritis over a period of five weeks before he came to the clinic. He suddenly became ill with fever, chills, nausea, vomiting and weakness. The fever disappeared and jaundice developed. Weakness rapidly progressed to exhaustion, stupor supervened and he died in coma twelve days following the onset of the illness.

At autopsy the liver weighed 1320 gm. The color was reddish brown, and the organ was mottled with small areas of ochre yellow. The capsule was slightly wrinkled. The liver was soft and flabby. The cut surface presented mottling of red and yellow. In the yellow areas, indistinct lobular markings were visible.

Microscopically a picture of nearly complete lobular disorganization was presented. Lobular units existed with a small amount of hepatic parenchyma present about the interlobular connective tissue or extending into the lobule up to two-thirds of the distance from the periphery to the central vein. In some lobules only the framework was preserved, for complete parenchymal dismantling of the lobule had occurred. The cellular detritus of the disintegrated hepatic tissue had been entirely cleared away. The initial toxic influence apparently had been directed against the hepatic parenchyma solely, with cellular necrosis and lysis of the cellular remains. The connective tissue, vascular apparatus, and biliary ducts apparently had not been involved in this process, for they persisted without observable reaction. The skeletons of lobules were easily identified by their intact interlobular zones, their sinusoids and central veins. Congestion existed in the bare sinusoidal areas. Frequently congested sinusoids appeared as bands extending between preserved units of parenchyma and connecting the portal and intralobular veins, or extending between two adjacent intralobular veins. Compared with the normal condition these sinusoidal structures were collapsed, but they still constituted the main means of transport of the sinusoidal circulation. The sinusoids about preserved hepatic cords contained but little blood. For the most part the cytoplasm of the hepatic cells was poorly stained; it appeared to be finely granular, with vesicular nuclei containing chromatin in finely divided particles. Completely intact cords were rarely seen. Isolated islands and portions of cords were the rule. Where only an eccentric rim of hepatic parenchyma existed the hepatic cords were atrophic and resembled bile ducts, the so-called pseudotubuli. Where these structures were cut in longitudinal section they appeared as swollen

any lipoids in the cells. In the partially preserved cells, fine, yellow granules of bilirubin were also seen. From some of the lobules complete disappearance of the cells had taken place, apparently by lysis. Where this process had taken place the hepatic reticulum and sinusoids persisted and were unchanged. When the lobule became completely devoid of hepatic cells, shrinkage occurred to about half the original size, but where the autolytic process had just been completed, the space occupied by the former hepatic trabeculum could still be seen. Later the space collapsed; the lobular portion was thus decreased in size. In the regions which were devoid of hepatic parenchyma, sinusoids were congested. Microscopic studies disclosed that the grossly red areas represented the skeleton lobules with congested sinusoids, and the grossly yellow portions were from the persisting or necrotic hepatic parenchyma.

*Comment:* This case exhibits clearly the early stage of hepatic necrosis and cytolysis resulting from intoxication by cinchophen. Cellular detritus was still largely persisting.

CASE 3. A woman, aged 57 years, had taken oxyliodide for rheumatism at intervals for five months. The drug had been taken in three short series, always with the appearance of toxic manifestations such as pruritus and loss of appetite. Finally the fourth trial was persisted in, even though evidence of toxicity reappeared as before. Jaundice finally developed and persisted. Vomiting, weakness, mental lethargy, and finally coma supervened, with death twenty-nine days after the onset of jaundice.

At autopsy the liver weighed 640 gm. It was reddish brown and was mottled by small, slightly raised, yellow granules which showed through the wrinkled capsule. The organ was leathery and somewhat flabby. The cut surface was reddish brown, and small dark green to pale yellow, indistinct, lobular mottled markings were distributed throughout all lobes.

Microscopically the retrogressive features were seen distinctly to be at an end, except for tiny foci of fresh necrosis which existed in some intact parenchymal units. Detritus of the former cytolytic action had entirely cleared. The persisting parenchyma was in the form of units of irregular shape and size, without regard to anatomical lobular demarcation. In some part of their periphery, these units were connected with the interlobular vascular and biliary apparatus, but rarely, if ever, were the central venous regions completely encircled by parenchyma. Central portions of lobules were frequently of triangular shape, due to compression of the bare sinusoidal structures by eccentric ingrowths of hepatic cords, beginning from three separate interlobular zones and crowding the region of

in the initial hepatic reaction. Repair was held in abeyance by persisting toxic effect or other factors unfavorable to the growth of hepatic cells.

CASE 2. The clinical features of this case were reported by Stacy and Van-zant. The patient was a woman, aged 52 years, with a history of carcinoma of the uterine cervix that had been treated with radium. Pain developed from metastatic carcinoma which involved the right sciatic nerve, for relief of which she took cinchophen. The drug had been taken for about six weeks, one to three tablets daily, when evidence of toxicity appeared. Death occurred sixteen days after the development of jaundice.

At autopsy the liver weighed 903 gm. Its color was red, with yellow, granular, slightly elevated mottling showing through the wrinkled capsule. The organ was soft, and flabby. The cut surface presented similar areas of red and yellow mottling; the yellow areas apparently more clearly demarcated hepatic lobules.

The nature of the initial hepatic lesion was best indicated by the microscopic appearance of the liver in this case. The process was directed against the hepatic parenchyma almost solely, without involvement of the reticulum, vascular apparatus or bile ducts. The toxic effect on the liver resulted in necrosis of the hepatic parenchyma, without evidence of exudative inflammatory reaction. The severity of the initial reaction apparently determined the extent of necrosis, and likewise the capacity of the liver to recover. In this case, a completely unaffected lobule never was found, and in most instances the entire parenchyma of given lobules was destroyed. It was difficult, for this reason, to state that the initial necrosis was central, peripheral, or intermediate in position. Wherever preserved hepatic cells existed, they appeared to be in close connection with the afferent blood supply, a fact which indicated that at least the beginning necrosis was probably not in the periphery of the lobule. The morphological evidence of necrosis was shown by the granular cytoplasm of the cells which took the eosin stain, so that they appeared as ghost forms, usually with complete absence of the nuclei, or with nuclei in a state of karyolysis. In the eccentrically placed, partially preserved units, evidences of degenerative cellular reaction existed, but with some indication that recovery of these cells might have occurred if the patient had survived. The cytoplasm of these cells was likewise pale, took the eosin stain, and was vacuolated, but the nuclei showed little degenerative effect. With scharlach R these vacuoles stained red, but in the necrotic portions there were scarcely



she had had complicating toxic hyperemesis. For the postpartum debility and pains she took freely of various analgesics, chiefly amidopyrine (pyramidon) and atophan. Jaundice and mild gastro-intestinal symptoms became manifest after about one year of this indiscriminate usage of drugs and finally manifested itself as icterus gravis. She died about fifteen and a half weeks after the onset of jaundice.

At autopsy the liver weighed 1045 gm. The color was yellowish green with depressed streaks and patches of brownish red. The capsule was smooth, but slightly wrinkled. The organ was firm, leathery, but somewhat flabby. The cut surface was mottled red and yellow. The red was accentuated in the left lobe. Lobular markings existed only in the yellowish portions and were of irregular size and shape. There was edema of the legs, and ascites with 2000 cc. of fluid.

Microscopic examination revealed considerable parenchyma. Often completely preserved or newly formed lobular structures existed. In general, however, the picture presented was one of dissociation with marked irregularity in the size and shape of the parenchymal groups. Frequently bands of fairly vascular connective tissue (the bare sinusoids) were placed between lobules, or extended through the centers of the lobules, dividing them into two or more apparently independent structures. Sometimes the preserved parenchyma was only in the form of a group of hepatic cells, with considerable separation of individual trabeculae by compressed sinusoids or their connective tissue basement membranes. Central veins were more frequently bare than were the portal veins. The regions that were almost completely devoid of hepatic parenchyma and also those that exhibited parenchymal atrophy about the portal structures gave evidence of the presence of peripherally placed tubules in intralobular position. These tubules sometimes completely surrounded the lobule and in their atrophic and compressed state appeared as bile ducts, having but slight resemblance to hepatic cords. The hepatic cells possessed pale, faintly granular cytoplasm containing granules of bile pigment. The cells contained very little lipoid. There was very slight lymphocytic infiltration in the portal connective tissue or in the connective tissue of the bare lobules.

*Comment:* This was a late stage of atrophy, exhibiting characteristics of early cirrhosis, and unmistakably pathogenetically related to the cases of shorter duration, previously described. Regeneration was not clearly evident. The ascites illustrated that it may occur even in the early cases of cirrhosis, its formation indicating portal

the intralobular vein before the growth. From the central position, bands of congested sinusoids sometimes extended so as to split a lobule between two adjacent intralobular veins, or between the central and portal veins. Evidence existed here, as in Case 1, that most of the sinusoidal circulation passed through these portions, rather than through the narrow capillaries between hepatic trabeculae. These parenchymal units of incompletely restituted lobules evidently were the beginnings of the hyperplastic nodules seen in the later stages. The cytoplasm of the hepatic cells was poorly stained, somewhat hyaline, granular and contained yellowish green granules of bilirubin. Bile thrombi were numerous in the bile canaliculi; sometimes the thrombi were so large that pseudoductal structures were formed by compression of the hepatic cords around the inspissated bile. The nuclei of hepatic cells were pale or vesicular, with finely divided chromatin. In some cells two nuclei were seen, but they were never in a state of mitosis. In the zones of complete parenchymal dissociation (the grossly red portions) there were clusters of eccentrically placed tubules. The appearance here more closely resembled that of bile ducts than in cases previously described. These tubules were not necessarily in relation to interlobular bile ducts but were found extending about the periphery of the lobule wherever normal trabeculae were lost. Variations in form, sometimes resembling hepatic cells and sometimes bile ducts, were evident in them. Many of them contained bile thrombi. There was but little evidence of lipoids in any of the hepatic cells. Lipoid was sometimes stained by scharlach R in the bare, sinusoidal portions where it either lay free, or in the endothelial cells. The connective tissue, as in earlier cases, consisted of persisting former hepatic framework, more contracted and apparently with some new cells derived especially from the sinusoidal endothelium. A faint pink reaction to the Van Gieson stain was for the first time evident in it. Leukocytic infiltration was no more prominent than in cases previously described.

*Comment:* This case represents maximal hepatic atrophy from intoxication by cinchophen, and illustrates an intermediate stage in the evolution of toxic cirrhosis.

CASE 4. Clinical features of this case have been reported by McVicar and Weir. A woman, aged 37 years, following the birth of a child had experienced general disability with rather vague aches and pains. In the course of pregnancy

so as to compress the surrounding cells. The connective tissue was light pink by Van Gieson staining. It gave evidence of considerable contracture and irregular compression, but still could be identified as of former hepatic derivation. There seemed to be some proliferative activity in the endothelial cells, slight in comparison with the amount of connective tissue present. There were a few leukocytes of mononuclear and polymorphonuclear types infiltrating the connective tissue, as in previous cases.

*Comment:* Early toxic cirrhosis is manifested in this case, with regenerative nodules in beginning formation. Nodules appeared as hypertrophic clusters of hepatic cells from previously preserved intact units. The abbreviated, peripherally placed tubules (hepatic cylinders) had been subjected to the same hypertrophic influences and appeared active rather than regressive. Their morphology more closely approached that of the hepatic cell than that of the cells of the bile ducts, and further, they appeared to have well defined hepatic function in metabolism of fat, and excretion of bile. The connective tissue was but slightly newly formed. It was still possible to determine the framework and sinusoids of the former lobules. The development of ascites indicated the degree of intralobular sinusoidal shrinkage, resulting in portal obstruction.

## DISCUSSION

The pathological changes produced by the toxicity of cinchophen, as shown in our cases and in those previously reported by others, are most clearly manifested in the liver. Other lesions, such as fatty changes in the heart and kidneys, mild fat necrosis of the pancreas, and mucosal and serosal hemorrhages were seen, but by comparison they were insignificant. These associated lesions were probably not primarily induced by cinchophen but were secondary to the toxic disturbances concomitant with the atrophy of the liver.

These cases further demonstrate that the hepatic lesion closely, if not exactly, duplicates the picture of acute and subacute atrophy or toxic cirrhosis of the liver, as produced by other causes. The exact picture is apparently determined by the severity and completeness of the initial reaction which either terminates in early death from rapid atrophy of the liver, or is prolonged into definite cirrhosis. From clinical deductions it is evident that less than fatal reactions

obstruction through contracture and shrinkage of intralobular sinusoids.

CASE 5. A man, aged 62 years, had taken oxyliodide for relief of arthritic pain. Toxicity soon became manifest as anorexia, weakness and loss of weight, and after two weeks jaundice developed. It cleared when taking of the drug was stopped, only to recur with persistence in its use. Finally grave icterus supervened in which the patient died about five months after the initial onset of jaundice.

The liver weighed 1134 gm. The color was brownish red, mottled by yellow, slightly elevated nodular areas varying from 1 to 5 mm. in diameter. The organ was firm and leather-like. The capsule was wrinkled. The cut surface had a mottled red and yellow appearance like that described for the capsule. There was ascites with 2000 cc. of fluid. (See Fig. 1.)

Microscopically the disorganized state of the liver was apparent, but former detritus of the regressive phase had cleared away, and the hepatic parenchyma appeared as revitalized. Well formed parenchymal nodules were found composed of large hepatic cells in widened trabeculae clustered together and either surrounding, or lying adjacent to, the interlobular portal units. Central veins were mostly bare, or they were approached by hepatic tissue on one side, appearing as if peripherally placed with regard to the hepatic unit, but in reality the veins were displaced to the periphery of the former lobule by compression from the hypertrophic hepatic nodules. In the hepatic lobules still existing as skeletonized structures, the peripherally placed rim of tubules was seen as in other cases. The same structures were evident about portal spaces where partial lobular denudation had occurred. These had quite a distinct appearance of abbreviated hepatic cords with cells in a hypertrophic state, as in the nodules of parenchyma. Their bile canaliculi were often shown by the presence of bile thrombi and they contained lipoids as frequently, in fact, as the undoubted hepatic cells contained them. In some places a point of union between these miniature hepatic cylinders and intralobular connecting bile ducts was seen. It was thus possible to see that the two structures were abruptly dissimilar. Gradual transition from one to the other did not exist. At once, as the eye travelled from the hepatic cylinders to the intralobular bile ducts, the cells became low cuboidal with rounded, large, basally placed nuclei. In some places the tubules possessed morphological similarity to bile ducts, especially when they appeared atrophic or when their canaliculi were filled by bile

ation and necrosis of hepatic parenchyma, with subsequent clearing of the detritus by autolysis and by the phagocytic action of leukocytes attracted into the field by the necrobiotic cells. The necrosis apparently develops and clears without injury to the blood vessels, connective tissue or bile ducts. Since the connective tissue survives this toxic insult without injury, it subsequently does not react to form new connective tissue, and thus the part it plays in the evolution of toxic cirrhosis is only passive. The connective tissue which constitutes the reticulum, sinusoidal structures and larger veins, persists and shrinks, an observation which Mallory made in his early descriptions of the lesion and which more recently has been reemphasized by Herxheimer.<sup>31</sup>

In the pathogenesis of toxic cirrhosis, the outstanding characteristics thus become: (1) relatively rapid necrosis and autolysis of the hepatic parenchyma resulting in atrophy of the liver; (2) relative increase of connective tissue which arises from the parenchymal loss, without injury or proliferative reaction on the part of the connective tissue framework or vascular apparatus of the liver; (3) predicated on the duration of life following the initial atrophy, regeneration will ensue, arising as reformed nodules of hepatic parenchyma from existent parts spared by the initial necrosis. Because of the extensive initial destruction, this regeneration will be extremely irregular and patchy, but nevertheless large nodules may occur.

The series of cases which we have reported adequately fulfills these three pathological characteristics. In the researches of other investigators added proof may be found. The hepatic lesion caused by cinchophen usually has been referred to simply as acute or subacute atrophy. Rabinowitz, however, recognized the similarity between the hepatic lesions produced by cinchophen and those arrived at through the effects of other etiological factors. He indicated that from cinchophen, as from other agents, the resultant lesion depended on the relative amount of atrophy, regeneration, cirrhosis and nodular hyperplasia, and that with longer duration, opportunity for regeneration with cirrhosis and nodular hyperplasia would be given. Reichle referred to the lesion in his cases as toxic cirrhosis. Parsons and Harding concluded from the lesions of the fifteen fatal cases which they had reviewed that acute, subacute, or chronic hepatic degeneration could result. A careful analysis of our cases should be of value in setting forth clearly the essential similarity of the toxic

may exist which end in recovery, but whether the restoration to clinical normality signifies complete anatomical and functional restoration or restoration in subclinical cirrhosis, can be judged only by future observation in these cases as the patients eventually may succumb from this or other causes. Since the so-called catarrhal types of jaundice are probably on the basis of mild intrahepatic toxic disturbances, some indication already exists that complete restitution, both anatomically and functionally, may occur. On the other hand, Eppinger<sup>28</sup> has shown by biopsy that from catarrhal icterus there may be progression to cirrhosis. This fact also coincides with the well known clinical problem met with in the idiopathic or genuine types of atrophy of the liver; they may appear to pursue a course characteristic of simple jaundice, only to exhibit an unlooked for termination in icterus gravis, and at autopsy the characteristic anatomical picture of subacute atrophy or toxic cirrhosis may be seen.

Marchand<sup>29</sup> in 1895 was probably the first to point out that in the idiopathic types of atrophy of the liver a relatively slowly progressive illness might ensue, terminating months rather than days or weeks subsequent to the onset, and showing in place of the lesion typical of acute atrophy of the liver one in which large nodules of hyperplastic parenchyma alternated with regions of red atrophy. He indicated that this lesion represented multiple nodular hyperplasia, on the basis of initial acute atrophy of the liver, and he set forth further that the resultant lesion was a type of cirrhosis. Mallory<sup>30</sup> in 1911 showed the pathogenetic relationship between acute atrophy of the liver and a specific type of cirrhosis, to which he gave the designation "toxic cirrhosis." He included this in his study as one of the five ways by which cirrhosis might arise. Similar conclusions have been reached by many other investigators.

The persistent concept of the inflammatory nature of all cirrhotic lesions of the liver, implying overgrowth of connective tissue, with compression of the regenerated lobules into nodular formations, has detracted considerably from clear understanding of the initial lesion and its subsequent development into toxic cirrhosis. As far as toxic cirrhosis is concerned, inflammation, excepting in the restricted sense of reaction to injury, is neither the cause nor the outstanding characteristic of the lesion. The reaction to the toxic substance in the early retrogressive period is in the form of fairly specific degener-

ness became more apparent, the regions appeared relatively more cellular. At times, especially in Cases 4 and 5, actual increase in the sinusoidal endothelial cells was thought to have occurred. The evidence of the reticular and sinusoidal character of the connective tissue was best demonstrated by use of impregnations with silver, such as are revealed by the Perdrau method (Fig. 5). Specific connective tissue stains, for the most part, failed to demonstrate fibrous or collagenous connective tissue, except in the interlobular regions, where the old connective tissue of the liver was unaltered. Thus was substantiated, by these special methods of staining, the distinctive, mainly reticular character of the connective tissue. Elastic tissue stains revealed its presence only in the walls of the blood vessels and the capsule. With regeneration of portions of lobules preserved from the initial necrosis, the sinusoids and reticulum became more evidently compressed between growing parenchymal nodules (Fig. 8) or at their rim. Partial separation of the new and old lobules, by such bands, was seen where they frequently extended between central and portal vein, or between central and sublobular vein. Bands of connective tissue formed of original reticulum were thus seen, dividing lobules into sublobular divisions, with the central vein apparently lying at the periphery of the parenchymal unit. The center of the lobule had in reality not been changed, but regrowth of parenchyma was exceeded by contraction of the connective tissue so that the central vein, lying in the banded, condensed reticulum, appeared to be placed eccentric to the lobule. Even in the late cases, when the contraction changes were most evident, most of the sinusoidal circulation seemed to be passing through these bands rather than between the hepatic cords.

If any differences exist between the lesions produced in intoxication by cinchophen, and those of other etiology, it is in the relative retardation of regeneration in the lesions produced by cinchophen. Grossly nodular formations were not observed even in Case 5, of five months' duration. Comparative studies of cases of toxic cirrhosis of unknown etiology (idiopathic) have shown much greater regenerative capacity in cases of the same and of shorter duration. This inhibition may have been the result of persisting toxic effect, or of other factors not yet definitely known. The duration of the lesion may have been more accurately estimated in the known toxic types. If this were true, then the differences were more apparent than real.

changes from cinchophen and those produced from other previously noted agents, such as chloroform and unknown toxins which induce the so-called genuine, or idiopathic hepatic atrophy.

The acute, or necrotic phase of the lesion was best illustrated by Case 2. The universality of the necrosis was the outstanding microscopic picture (Fig. 2). A completely intact lobule could not be found in any of the sections examined. The cells appeared to become necrotic without interposition of fatty changes, and from necrosis they appeared to melt away without provoking inflammatory reaction excepting that a few leukocytes invaded the field, probably as an adjunct in clearing up the detritus left behind. In an earlier stage, preceding the necrosis, the cells may have contained more fat. The anatomical starting point of the necrosis was probably central, for the only preserved parts of the lobules existed in close connection with the portal veins and hepatic arteries. In the other cases this initial reaction had apparently come and gone, for evidences of similar necrotic lesions, even in part, could not be observed in them. By comparison of Case 2 with other cases we might presume that necrosis was rapid, progressive to a certain stage, and ended sharply. Detritus was rapidly cleared, leaving disorganized hepatic cords and lobules behind (Fig. 3). Since life was spared longer in these cases, the initial reaction had undoubtedly been less severe. A greater amount of parenchyma was preserved. As in Case 2, however, the preservation was always best illustrated in the eccentric portions of the lobules (Fig. 4).

The second phase of toxic cirrhosis concerns the increase in connective tissue. Changes described for toxic cirrhosis in general were also met with in our observations when applied to the connective tissue phase. When the necrotic cellular detritus had cleared, the sinusoids and their supportive reticulum could be seen unaltered, except for loss of hepatic cords. Even where the entire lobule represented but a skeleton of its former self, all parenchyma having disappeared, the preservation of this sinusoidal architecture was easily identified (Fig. 6). The spaces formerly occupied by the hepatic cords were clearly evident in Case 2. In some regions in the same case, but better shown in other cases, these spaces were collapsed (Fig. 7). Later, sinusoids themselves collapsed through continued shrinkage, until fairly compact lobular units composed of only a few open and congested sinusoids were found. As the greater compact-



rebuilding of hepatic tissue, as revealed in toxic cirrhosis of cinchophen derivation and in other types, for they regress with the advancement of reticular and sinusoidal shrinkage, before they become of functional importance. From them new lobules probably never form. Regeneration proceeds almost entirely from the better preserved hepatic units, as already indicated. Although in our cases of poisoning from cinchophen the regenerated tissue has never attained large nodular formations, in other cases of toxic cirrhosis nodules of irregular size, shape and distribution, arising from a generally atrophic red substance, constitute one of the outstanding characteristics of the lesion (multiple nodular hyperplasia).

The lesions of toxic cirrhosis, in their pathogenesis and in their completed form, are distinctive from those of the ordinary Laennec, alcoholic, or portal cirrhosis. Likewise, the clinical manifestations are distinctive in each. In the toxic types, a relatively more rapid course is pursued from onset to termination. The long incipient period of Laennec cirrhosis, with latency of clinical manifestations, is a distinctive characteristic, not manifest in the toxic types. The usually early appearance of jaundice, remaining quite constantly throughout the duration of the illness, is an almost invariable picture of toxic cirrhosis, whereas, in the ordinary Laennec form, jaundice is either never an accompaniment or usually appears only as a terminal manifestation. Ascites in toxic cirrhosis is a latent complication, but in the Laennec type the clinical onset of illness is frequently dated from its first appearance.

In toxic cirrhosis there is initial severe and relatively rapid destruction of hepatic parenchyma, similar to, but less decisive than that seen in typical acute yellow atrophy of the liver. The duration of life beyond this initial reaction is primarily dependent on the amount of hepatic parenchyma spared, and secondarily it is determined by the capacity of the hepatic cells to regenerate. The duration of life in the presence of severe parenchymal destruction predicates the extent to which actual cirrhosis will be found. The initial atrophy of the hepatic parenchyma in toxic cirrhosis is always complete in local regions, so that only widely scattered groups of lobules may escape. Since significant regeneration may proceed only from the well preserved groups of cells of former hepatic lobules, the extent to which it may occur is limited. The reformed lobules appear in clusters, usually widely separated from other groups by

As in the other forms of toxic cirrhosis, regeneration here, although limited, was seen to proceed from intact preserved portions of hepatic parenchyma (Figs. 9 and 10) which had escaped disorganization in the initial destructive lesion. The regeneration was through hypertrophy and hyperplasia of the hepatic cells in nodular formations. The cords were thickened, individual cells had enlarged, and often a syncytial arrangement was found in them. Nuclei were frequently double, but mitotic figures were almost never found. About the periphery of the lobules, where intact trabeculae were absent, or adjoining the interlobular connective tissue when atrophy and disappearance of the cords was found in that situation, tubular structures, sometimes resembling bile ducts, and sometimes resembling miniature hepatic cords, were seen. In Case 1 (Fig. 4) the dissociation of the hepatic cords appeared to follow in such a way that every gradation between these apparently newly formed tubules and the old, intact, but injured hepatic cords could be distinguished. In Case 5, the cells of these tubules shared in the hypertrophy and revitalization, previously described as being found in the larger preserved portions of hepatic parenchyma. In spite of this, the cells were still peripherally placed and gave no evidence of progression beyond the stage of tubules.

It is still held by many observers that these peripherally placed structures represent proliferated bile ducts; some even have gone so far as to believe that they constituted the chief units from which hepatic cells regenerated. Herxheimer and Gerlach,<sup>32</sup> and Blum<sup>33</sup> have observed them in a light quite similar to that which we have described; namely, that morphological evidence and intralobular position point to their derivation from retrogressing hepatic cords, even from the first, when retrogressive rather than regenerative changes dominate the picture. Further confirmation of their hepatic derivation existed on functional grounds, for they contained fat, bile pigment, and apparently excreted bile into structures which were like bile canaliculi (Fig. 11). When seen to connect with intralobular-joining bile duct structures, the cellular change was abrupt, never gradually transitional (Fig. 12), indicative of complete dissimilarity of bile duct cells and those of the tubules. With condensation of the connective tissue, they usually underwent atrophy; then they resembled bile ducts much more than hepatic cells, but still were usually distinguishable from bile ducts. They are insignificant in the

2. Unknown factors, apparently independent of the quantity of the drug used, appear to be significant in creating a predisposition or idiosyncrasy for the drug.

3. Various grades and stages of hepatic degeneration have been described. These are presumably dependent on the completeness and rapidity of the initial reaction. The reaction may be rapid and complete, with induction of acute atrophy of the liver, or slower and less complete, with apparent recovery. Intermediate between these two extremes subacute forms of intoxication may ensue and may become manifest both clinically and pathologically as a type of hepatic atrophy or cirrhosis, which corresponds in its anatomical characteristics to the distinctive toxic cirrhosis as described by Mallory.

4. The clinical and anatomical characteristics of toxic cirrhosis appear to be specific and essentially dissimilar to the ordinary Laennec or portal type.

5. The clinical data and correlated studies of pathological anatomy in five cases of intoxication from cinchophen constitute the basis for this study.

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portions completely devoid of these regenerative forms. In the Laennec form of cirrhosis the initial atrophy is apparently never as severe, and likewise it never occurs so rapidly. Thus, the regenerative nodules are more abundant and uniformly distributed, with less variation in size and shape, more nearly duplicating the former hepatic units. In the toxic types, at least, the connective tissue largely represents the preëxisting hepatic reticulum, sinusoids and interlobular partitions made prominent by the disappearance of the hepatic cords. Proliferative activity never plays a prominent part in this increase of connective tissue. The contracture and shrinkage of this original stromal substance parallels the contracture and hardening of the organ as a whole, augmented in part by the regenerative nodular forms. Later it may assume the characteristics of fibrous or collagenous connective tissue, as Rinehart<sup>34</sup> and others have indicated that from organic reticulum this transformation may take place. Even in the late cases of toxic cirrhosis, in the grossly red portions the skeletal structures of former lobules still persist, an identifying characteristic not seen in other types of cirrhosis. The inability to identify these skeleton lobular structures in Laennec cirrhosis, combined with the associated, almost constant and abundant lymphocytic collections in the evidently increased connective tissues, apparently indicate a difference in the pathogenesis of these two distinctive types of hepatic atrophy. The appearance of the so-called proliferated bile ducts in each type needs further study before a conclusive statement can be made. Apparently, in the toxic type, as we have indicated, their formation is from regressive hepatic trabeculae, and although they usually present progressive atrophy, sometimes they become activated by the same influences which govern the activation to growth of other hepatic cells. Not only, however, is this development hindered by the action of the primary toxic factor, but also by the progressive contracture of stroma which eventually literally crowds them from the picture, for they become contracted to small structures which resemble bile ducts or assume the appearance of mere strands of parallel cells.

#### SUMMARY

1. Preparations of cinchophen have been shown definitely to be toxic for certain persons. The toxic effects are directed most severely and specifically against the liver.

## DESCRIPTION OF PLATES

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### PLATE 46

- FIG. 1. Case 5. Toxic cirrhosis caused by cinchophen, of five months' duration.
- FIG. 2. Case 2. Extreme lobular disorganization and necrosis. Congested sinusoids. The best preserved unit is at the lower left, adjacent to the interlobular circulation (not included). Interlobular structures are at the right, midway between top and bottom; they are surrounded by bile ducts and intralobular sinusoids which are made apparent by parenchymal autolysis; congested central vein is at the upper left, surrounded by prominent sinusoids and reticulum. Hematoxylin and eosin stain.  $\times 65$ .
- FIG. 3. Case 2. Complete dismantling of lobule, with cellular detritus cleared. Some sinusoids and the central vein are congested, other sinusoids with reticulum are collapsed. At the upper left, a portal vein is surrounded by bile ducts made prominent by disappearance of parenchyma. Hematoxylin and eosin stain.  $\times 120$ .
- FIG. 4. Case 1. A less destructive lesion than those of Figs. 2 and 3, with portions of lobular trabeculae, regressive but simulating biliary duct structures. Actual bile ducts are prominent in the interlobular zone, upper left. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 5. The sinusoidal (intralobular) reticulum of normal liver as revealed by silver impregnation by the method of Perdrau.
- FIG. 6. Case 2. The intralobular sinusoidal structures and reticulum as revealed by silver impregnation by the method of Perdrau. Necrotic detritus is still persisting somewhat, but where clearing has occurred sinusoids are collapsed.  $\times 125$ .

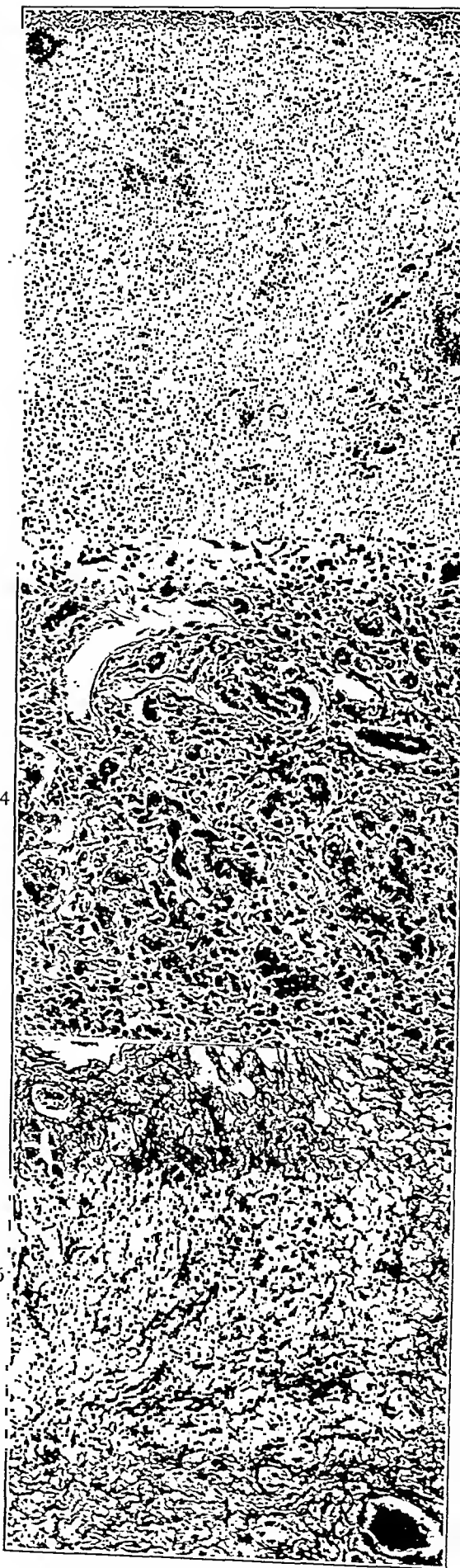
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PLATE 47

- FIG. 7. Case 4. Lobular reticulum in late atrophy as shown by silver impregnation, by the method of Perdrau. Lobular landmarks are evident: central vein, collapsed sinusoids, peripheral rim of tubular intralobular structures and interlobular structures, upper left.  $\times 110$ .
- FIG. 8. Case 5. Compression of skeletonized lobule by growing parenchymal nodules as revealed by silver impregnation by the method of Perdrau. A central vein is lying in compressed lobular stroma at the periphery of a regenerated nodule (not included) at the right.  $\times 110$ .
- FIG. 9. Case 3. Units of trabeculae with evidence of recovery from toxic effect, surrounding an interlobular zone, in which bile ducts are prominent. Pseudo-intralobular ductal formations are in the miniature hepatic cords formed by dilatation of bile canaliculi. Nodular regeneration proceeds from such partially preserved lobules as shown here. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 10. Case 5. Regenerated lobules in nodular formation. A band of hepatic reticulum is compressed between regenerated parenchymal units. Tubular structures consisting of bile ducts and regressive hepatic cords lie in original but contracted hepatic stroma and surround the newly formed lobules. An hepatic vein lies at the periphery of the regenerated lobule. Hematoxylin and eosin stain.  $\times 50$ .
- FIG. 11. Case 5. Tubular structures exhibiting bile thrombi, at the periphery of a lobule. The resemblance to hepatic cells in these structures is striking. Hematoxylin and eosin stain.  $\times 350$ .
- FIG. 12. Case 5. Peripherally placed intralobular tubular structures, showing abrupt transition between the apparent bile duct and the miniature hepatic cord. A characteristic bile canaliculus lies between the cell rows of the miniature hepatic cord. Granules of bile and lipochrome pigment are within the hepatic cells. Hematoxylin and eosin stain.  $\times 300$ .



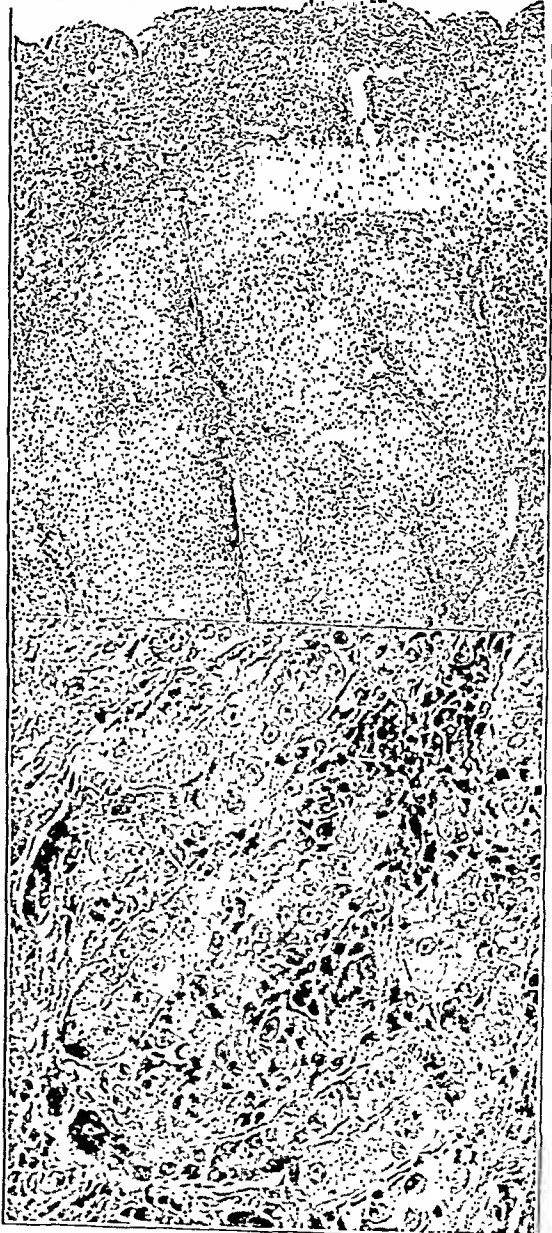
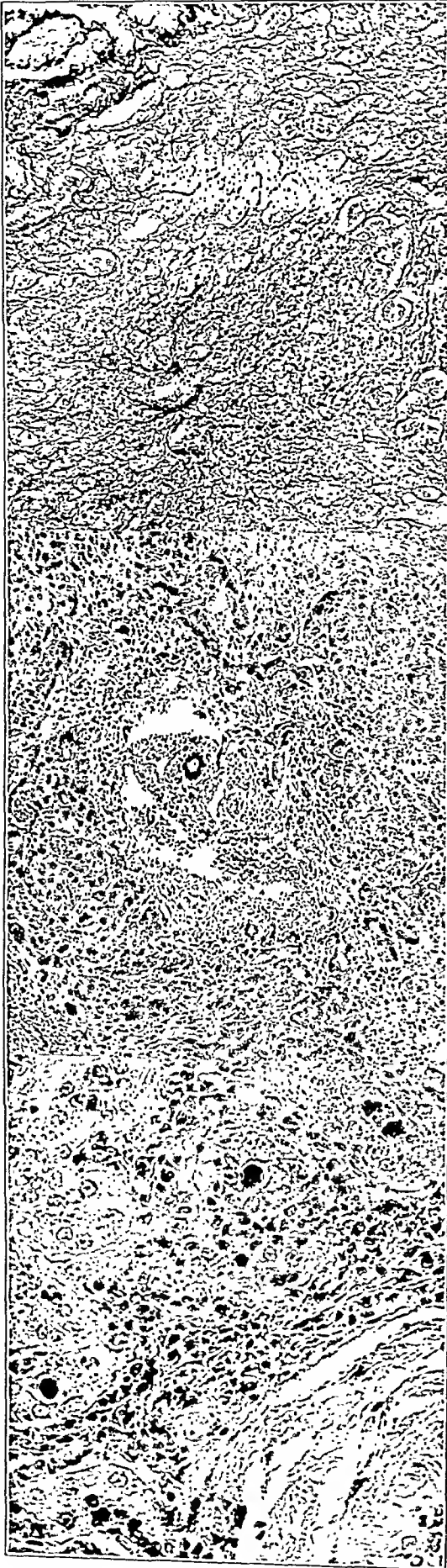
Beaver and Robertson



Toxic Cirrhosis in Cinchophen Poisoning







Beaver Robertson

Toxic Cirrhosis in Cinchophen Poisoning

special clinical significance, nor are they of long duration, since the patient dies of the generalized sepsis before attention is directed to the liver. There are instances in which superficial, localized infection appears to be the only source of an abscess of the liver and apparently produces its effect without first involving lungs or heart. Reiniger,<sup>1</sup> Kaufmann,<sup>2</sup> and others explained it on the basis of retrograde embolism and thrombosis of the hepatic veins. Such an occurrence, however, must be extremely rare. Small lesions could exist in the pulmonary parenchyma without detection and no doubt may constitute the focus for arterial dissemination, with localization in the liver.

Abscesses of the liver, which originate in the biliary tract, should be considered with diseases of the gall-bladder and the bile ducts. Ascending infection is not a probability unless obstructive lesions are complications, and then suppurative cholangitis may lead to formation of hepatic abscesses. Abscesses of this type are identified by their content of bile and their communications with the dilated biliary ducts.

Extension of infection from within the field of drainage of the portal vein is responsible for the greatest number of hepatic abscesses. This extension may occur by embolic masses of bacteria, but more frequently there is preceding local thrombophlebitis produced by the primary lesion, and the hepatic dissemination occurs as a result of detachment of infected thrombi. The original thrombophlebitis may extend to pylephlebitis with invasion of the liver and formation of abscess. The infrequency of localization of infection in the liver indicates that showers of infected embolic thrombi are almost essential predisposing factors in formation of hepatic abscess. Microorganisms unassociated with thrombi probably pass through the liver in most instances without producing suppuration.

The source of the hepatic infection may be apparent to clinical, as well as to pathological examination. These primary sources of hepatic infection are given by Rössle<sup>3</sup> in accordance with their frequency, as follows: colon, appendix, spleen, pancreas, small intestine and stomach. With the exclusion of amebic abscesses the appendix becomes the most common primary focus. In newly born infants infections of the umbilicus may proceed to thrombophlebitis of the umbilical vein, and with extension to the portal vein abscesses of the liver may supervene. Ulcerations of the rectum, or infected internal

## GRANULOMATOUS ABSCESS OF THE LIVER OF PYOGENIC ORIGIN \*

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Abscesses of the liver are relatively uncommon in comparison with the high incidence of other intra-abdominal infections. Nevertheless, there is opportunity for infection of the liver because it is in direct vascular communication, through the portal circulation, with most of the abdominal viscera. In addition, opportunity for infection with formation of abscesses is afforded by way of the hepatic artery, the bile ducts and the lymphatic channels. The protection of the liver against infection is to some extent dependent on its abundant blood supply, with free movement of blood through its sinusoidal vascular system. Further inhibition to localization of infection is afforded by the phagocytic reticulo-endothelial cells which line the sinusoids, and other less definite but none the less potent factors of immunity, in the production of which the liver is probably directly concerned.

### PATHOGENESIS OF HEPATIC ABSCESES

Abscesses which originate from infection carried by way of the hepatic artery are almost never found, except in a general pyemic process when the liver, with the other viscera, may suffer from dissemination of infective material. Before hepatic infection can occur from this source, pulmonary or cardiac involvement is almost a prerequisite. Microorganisms of sufficient virulence to infect healthy tissues usually will not pass the lungs without first localizing there, subsequent arterial propagation of the infection coming from the focus thus formed. Hepatic abscesses which originate in this way are multiple, small, and usually involve all lobes of the liver equally. Subcapsular localization is most common. These abscesses have no

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*Etiology:* A great number of microorganisms have been reported as playing the causal part in hepatic suppuration. My experiences concur with the reported observations of others in emphasizing the prevalence of pyogenic micrococci in this form of suppuration. Staphylococci predominate, with streptococci closely second. Other organisms occasionally constitute the exciting factor, such as members of the genus *Clostridium*. *Clostridium welchii* has been reported by others and we once found an organism corresponding to *Clostridium oedematis-maligni* (*Vibrio septique*). As a complication to infections with typhoid and paratyphoid organisms, hepatic abscesses have been reported, but their occurrence is rare. *Pseudomonas* and fusiform bacilli have been found rarely. The association of the actinomyces, streptothrix, blastomyces and entameba are well known. Infections by *Escherichia coli* in the liver, as elsewhere, probably have been overemphasized.

### CASE REPORTS

Several abscesses of the liver, having the characteristics of granulomas, have been seen at The Mayo Clinic. These usually have presented an insidious clinical onset, slowly progressing into chronicity without, as a rule, clearly revealing the source of the hepatic infection. Their drainage usually was unsatisfactorily completed because of their multilocular character and their tendency to persist as granulomas, with sometimes formation of sinus tracts. In their insidious progress, chronicity, cryptogenic character and anatomical features they have simulated the granulomas of actinomycosis or tuberculosis. The suspicion that either of these might have been the cause of the condition in these cases could never be confirmed. Bacteriological studies, on the other hand, have shown the constant presence of the pyogenic micrococci, both staphylococci and streptococci exhibiting this association. Thus, we may speak of these abscesses as pyogenic granulomas. The clinical aspects of hepatic abscesses will be presented briefly. Special emphasis will be given here to the duration, source, etiology and pathological anatomy. To illustrate the special characteristics of this disease protocols of eight cases will be presented.

CASE 1. A man, 44 years of age, complained of severe pain in the right upper abdominal quadrant, with nausea, chills and fever of three weeks' duration.

hemorrhoidal veins, have been recorded as other sources of portal infection. Rössle described two cases of splenic abscess with secondary hepatic infarction and suppuration. Direct, penetrating trauma to the liver may easily provoke formation of abscess, but more interesting are the observations of Townsend<sup>4</sup> and Jacoby<sup>5</sup> that severe contusions to the liver apparently evoke the local lowering of resistance which allows infection, with formation of abscess, to follow.

In some cases the hepatic suppuration appears to be the primary clinical phenomenon, and no evidence can be found by the pathologist to alter this opinion. Because of the apparent primary character of such abscesses they have been referred to as primary hepatic, idiopathic, or cryptogenic. It is unlikely, however, that the hepatic abscess ever represents primary infection, but more likely that the primary lesion is obscure, usually within the region of the portal drainage, and without subjective or objective signs, thus escaping the attention of both physician and patient. Healing may be so effectually consummated that even by careful examination the pathologist cannot give the needed help in offering a solution after death of the patient. Abscesses of this type have been referred to by Norris and Farley,<sup>6</sup> Lepehne,<sup>7</sup> Ludlow<sup>8</sup> and Williamson.<sup>9</sup>

Abscesses resulting from a source known to be primary in the tissues contributing to the portal circulation are most frequently found in the right lobe of the liver. Sérégé,<sup>10</sup> by means of injections of Chinese ink, seems to have offered an explanation for this. He showed that two currents of blood exist in the portal vein, one originating from the superior mesenteric and pancreatic veins, and passing to the right lobe; the other coming from the inferior mesenteric and splenic veins, and passing to the left lobe. Since the primary foci are most often thought to be in the distribution of the superior mesenteric vein, and if the observations of Sérégé are true, this would seem to explain the greater frequency of involvement of the right lobe.

Most of the so-called idiopathic abscesses have similar right lobar distribution and otherwise partake of the same anatomical characteristics as those of known portal pathogenetic relationship, even to the occasional association of pylephlebitis. These points of similarity suggest, as one would suspect, that their pathogenesis is the same, only our inability to localize the original lesion constituting the difference.

lar degeneration, progressing to necrosis as the region of infection was approached. In this zone thrombosis of the hepatic and portal veins was observed, with purulent infiltration of the thrombi and purulent phlebitis. Within the zone of encapsulating hepatic tissue there was fibrosis, which showed evidence of old and recent hemorrhage, and which was infiltrated with large mononuclear and polymorphonuclear leukocytes. Within this, and adjacent to the field of suppuration there was a zone of hyaline material which took the eosin stain, and into which a scaffolding of fibroblasts was penetrating. The exudate was predominately of polymorphonuclear leukocytes, and much necrosis was evident. Colonies of bacteria which resembled actinomyces were present, but with higher magnification these were revealed as staphylococci. The interlacing connective tissue trabeculae formed the partitions seen in the abscess cavities and represented the proliferated hepatic stroma. Bile ducts persisted in these strands.

*Staphylococcus aureus* was isolated in cultures from the hepatic abscess. Gram-Weigert stains of the preparations of tissue revealed many Gram-positive micrococci.

*Comment:* This case of chronic hepatic abscess is illustrative of an intermediate stage of progression with incomplete solution of the hepatic substance (Fig. 2), and some features of early granulomatous change. The etiological relationship to the peritoneal infection following perforated duodenal ulcer appears to have been well established.

CASE 3. A boy, 14 years of age, entered The Mayo Clinic complaining of swelling and tenderness of the left cervical region and right cheek. The cervical condition had been present, with alternating periods of healing and suppuration, for about two years. Six weeks prior to his admission, he had become suddenly ill, with dizziness, coryza, non-productive cough, fever, vomiting, and slight jaundice. Study of the suppurative process in the neck failed to give evidence either of tuberculosis or actinomycosis. Blood cultures were negative. He had an irregular fever, with daily fluctuations from 97° to 103° or 105° F. Abdominal exploration revealed an hepatic abscess, and drainage was instituted. Death occurred twenty-seven days after operation and about three months subsequent to symptoms referable to infection of the liver.

An autopsy was performed. The liver weighed 3085 gm. There were multiple multilocular abscesses involving the right lobe of the liver. Smaller ones, formed by peripherally extending infection from the primary hepatic foci, were seen. The largest abscesses were of irregular leaf shape, as described by Kaufmann, and were composed of well defined multicentric foci. Actinomycosis was strongly suggested by their appearance, as well as by the history of cervical suppuration.

Abdominal exploration revealed an enlarged liver. An hepatic abscess was found and drainage was instituted. Death occurred about five weeks from the onset of symptoms.

At autopsy the liver weighed 1948 gm. There were multiple multilocular abscesses which involved only the right lobe. The original hepatic focus was of central situation, with some peripheral extension. There was associated suppurative thrombosis of the portal and splenic veins. A small abscess 3 cm. in diameter was situated in the head of the pancreas and communicated with the thrombosed splenic vein.

Microscopically it could be concluded that the multilocular cavities were comparatively independent in structure because of the encapsulation of each small unit by compressed hepatic parenchyma. Adjacent to the exudate, the hepatic cells gave evidence of hyaline degeneration of the cytoplasm and other stages of retrogression to necrosis. Some fibroblasts were present in the capsule. The exudate was of polymorphonuclear leukocytes and clumps of bacteria were prominent.

Cultures taken from the hepatic abscess revealed *Staphylococcus aureus* and streptococci of a hemolytic type. Gram-Weigert stains of the tissue revealed many Gram-positive cocci in chains and clusters.

*Comment:* The only focus of infection found was the pancreatic abscess. This, however, could have been secondary to the pylephlebitis. Since this case presents a multilocular abscess (Fig. 1), associated with known thrombopylephlebitis, it supports the theory that these abscesses are of portal embolic origin, whether frank portal thrombosis exists or not.

CASE 2. A man, 47 years of age, entered The Mayo Clinic with an acutely perforated duodenal ulcer and consequent peritonitis. Symptoms of ulcer had been present for twenty years. The perforation was closed but the course of the disease continued to be septic, with irregular fever and progressive weakness. Death occurred about two months after the perforation.

At autopsy the liver weighed 4080 gm. An abscess 15 cm. in diameter existed in the right lobe of the liver, involving the inferior and posterior portion and extending laterally. It contained 1200 cc. of thick, greenish pus, but this was not bile-stained. The abscess was crossed by interlacing trabeculae separating it into several large cavities. The main wall of the cavity consisted of compressed hepatic tissue of grayish color, with some fibrosis. Thrombi were not observed in the portal veins.

Microscopically the outer part of the wall of the abscess consisted of compressed hepatic parenchyma showing varying stages of cellu-



abscesses with empyema. Recent thrombosis of the internal iliac veins, vena cava and right auricle also were found.

Microscopically the small foci of more recent formation gave evidence of their thrombotic nature, for suppurative thrombi were contained in partially intact portal veins. In the most recently formed foci there was no proliferative reaction about the periphery, but those of longer duration and representing the original hepatic foci of infection had walls composed of recently formed granulation tissue. Endothelioid and giant cells also constituted a part of this wall. Farther removed from the field of infection the encapsulating connective tissue was of more fibrous type. The exudate was composed of polymorphonuclear neutrophilic leukocytes combined with many large and small mononuclear cells, and in some fields there was hyaline necrotic substance with very few leukocytes present. Colonies of micrococci were present everywhere in the exudate. Surrounding the larger abscesses the hepatic tissue was compressed with central lobular congestion and necrosis.

Cultures taken from the hepatic abscess contained *Staphylococcus aureus*. Gram-Weigert stains of the tissue revealed clusters of Gram-positive staphylococci.

*Comment:* The origin of this hepatic infection could not be well established. The tip of the appendix was slightly thickened and infiltrated with lymphocytes, but did not present changes such as should have been found in a quiescent interval after a severe, preceding acute inflammatory process. However, no other explanation was revealed by the postmortem studies.

CASE 5. The patient was a man, 32 years of age. Five years before his registration at the clinic he had undergone appendectomy, and two years after that operation, hemorrhoidectomy. The illness of which he complained had begun with a sudden, constant, severe sacro-iliac pain. Later, intermittent epigastric pain had developed and this had become localized in the lower part of the abdomen. Subsequently pain in the right side of the thorax, jaundice and high fever had developed. Laparotomy revealed an abscess of the liver and drainage was instituted. The patient died about seven weeks after the hepatic drainage, and four months from the inception of the illness.

At autopsy the liver weighed 2500 gm. It was intensely stained with bile. There was no obstruction to the ducts or any cholangitis. Two multilocular abscess cavities were present in the right lobe, one on the anterior surface and one in the inferior portion. The inferior abscess measured 10 cm., 4 cm., and 3 cm. in various diameters, and the anterior one was 5 cm. in diameter. Surrounding the large abscesses smaller similar lesions were developing. The ab-

There was no pylephlebitis. 2000 cc. of ascitic fluid were found, and there were 700 cc. of fluid in the right side of the thorax. There were multiple embolic abscesses in the right lung.

Microscopically, there were well defined granulomatous encapsulations about each focus of the multilocular abscesses with suppurating centers consisting chiefly of polymorphonuclear leukocytes in which necrosis was evident. Colonies of Gram-positive cocci were abundant in the exudate.

Cultures of the hepatic and cervical abscesses contained *Staphylococcus aureus* and streptococci of a hemolyzing strain. Gram-Weigert preparations of the abscesses of the liver revealed numerous colonies of micrococci. No trace of actinomyces could be found.

*Comment:* This case represents a typical portal embolic abscess (Fig. 3), in spite of the cervical suppuration preceding it. There was no evidence of a retrograde embolic process in the hepatic veins. The source of the hepatic infection was probably in the portal circulation in spite of our inability to find it at the time of the autopsy. However, it is possible to follow events from cervical suppuration to pulmonary involvement and localization in the region of the portal circulation with secondary hepatic emboli supervening. The chronic suppuration in the neck led further to the suspicion of the presence of tuberculosis or actinomycosis, but confirmatory evidence for this could not be discovered.

CASE 4. A man, 27 years of age, complained of fever and weakness of two months' duration. This started as a sudden pain in the right lower part of the abdomen, and a diagnosis of appendicitis was made by his physician at home. Four days later, fever became marked, and continued with a somewhat irregular course. At laparotomy a subphrenic abscess was drained. Subsequent to the drainage, as before, the temperature continued to be of the characteristic septic type of hepatic suppuration, varying from 97° F. in the morning to 105° F. in the afternoon with frequent chills. He died two weeks subsequent to the drainage, and about three months after the probable onset of the illness.

At autopsy, fibrous adhesions were found extending between the superior surface of the liver and the diaphragm. The liver weighed 2865 gm. On section, in the right lobe just above the hilum, several intercommunicating abscess cavities, some with multilocular architecture, appeared. These extended laterally, and all together involved a region of liver about 10 cm. in diameter. Small, single or multilocular cavities of more recent origin appeared in the peripheral zone, a considerable distance from the old region of involvement. The larger cavities contained thick, purulent exudate. The centers of many of the multilocular regions appeared to be caseous. Thrombosis of the portal vein was not evident. There were associated subphrenic infection and embolic pulmonary

The drainage persisted, and several months later evidence of hepatic suppuration developed. Hepatic drainage was then instituted. Search was repeatedly made in the material which drained from the thorax for actinomyces and *Mycobacterium tuberculosis*, but always without discovery of either. The patient died seventeen days after the liver was drained and ten months from the beginning of the illness.

At autopsy, the liver weighed 2715 gm. In the lateral portion of the right lobe, a spongy, multilocular abscess was found which measured 10 cm. in diameter. The individual abscesses which comprised this mass were not more than 5 mm. in diameter. Each contained thick yellow pus. The trabecular partitions of the abscess appeared as grayish, fibrous encapsulating membranes 1 to 2 mm. in thickness. The peripheral encapsulation of the entire mass did not differ from the honeycomb-like trabeculae, except that compression of the hepatic tissue had occurred at the border. Evidence of old, healed empyema on the right side, and terminal embolic pulmonary abscesses were additional disclosures. There was no pyelephlebitis. The distal portion of the appendix was bound down by fibrous adhesions and the lumen was obliterated. There was no evidence of recent inflammation.

Microscopically the encapsulations of the individual units of the large multilocular abscess appeared to be of the same duration. Their structure was of fibrous connective tissue, apparently originating from hepatic tissue, because occasionally atrophic bile ducts could be identified. Nearer to the zone of suppuration young fibroblasts and endothelial cells became the principal framework of the capsule. Here there was infiltration with mononuclear and plasma cells, and polymorphonuclear leukocytes. The periphery of the exudate appeared as a layer of fibrin with few leukocytes, invaded by fibroblasts. The exudate within this was predominatingly of polymorphonuclear leukocytes, but much of it was necrotic. Colonies of bacteria were numerous. The hepatic tissue about the periphery of the multilocular mass gave evidence of compression, fatty and hyaline cytoplasmic changes in the hepatic cells, with considerable atrophy and disarrangement of lobules.

Gram-Weigert stains of tissue revealed numerous colonies of staphylococci in the exudate.

*Comment:* This case illustrates a multilocular or honeycomb abscess like that seen in actinomycosis, persisting into chronicity without exhibiting even sufficient lytic qualities to break down the divisions of hepatic tissue which originally constituted the encapsulating membranes of the multilocular abscess. Stimulation to granulomatous formation rather than advancement of the suppurative characteristics was the outstanding anatomical feature of the hepatic lesion. The onset followed a questionable attack of appendi-

scusses were composed of large multilocular cavities with intercommunications. In some places the trabeculae had broken down to tag-like bands that were adherent to the walls. Fibrous encapsulation was evident. There were beginning subphrenic abscess and empyema on the right side. Chronic ulceration was present in the terminal portion of the ileum. There was no evidence of pylephlebitis.

Microscopically the hepatic abscesses appeared to be irregularly outlined and multiple. The encapsulating structure was of fibrous tissue lying on compressed hepatic substance. In the innermost portion of the wall, fibroblasts, blood vessel sprouts and giant cells were evident, and this zone was infiltrated by mononuclear and polymorphonuclear leukocytes. The exudate consisted of polymorphonuclear leukocytes, but in part it was composed of hyaline-like material mixed with cellular detritus. Colonies of bacteria were numerous in the exudate. Considerable necrosis and hemorrhage existed in the hepatic tissue adjacent to the abscesses. The ulcerative lesions of the ileum exhibited chronicity, and there were lymphocytic collections and endothelial proliferation throughout the intestinal wall. The ulcers did not have specific characteristics.

Cultures from the abscess of the liver revealed green-producing streptococci. Gram-positive streptococci were abundant in the tissue.

*Comment:* The insidious onset and chronicity of this hepatic abscess, together with the prodromal localization of pain in the lumbar and lower abdominal regions, constitute the outstanding characteristics. Williamson has indicated that the pain may be referred to the lumbar region from the diaphragm in subdiaphragmatic or hepatic suppuration. In the early stages of this case, typhoid fever, undulant fever and tularemia were suspected but could not be proved. The hepatic lesions at autopsy suggested actinomycosis, but actinomyces were never found. The probable source of the abscesses of the liver was a cryptogenic intestinal infection, with embolism to the liver. The residual of this may have persisted as ulcerative enteritis. The appendectomy or the hemorrhoidectomy, performed some years before, could be of significance as constituting the primary focus, but the interval of good health following these procedures renders this improbable.

CASE 6. A man, 31 years of age, whose illness began as appendicitis, subsequently had chills, fever, nausea, vomiting and pain in the right side of the thorax. Empyema of the right pleural cavity developed which was drained.

Exudate from the hepatic abscess revealed staphylococci in smears, cultures, and in preparations of fixed tissue. Actinomyces were not found. *Mycobacterium tuberculosis* was not found in smears.

*Comment:* This case is representative of the so-called idiopathic hepatic abscess. There was an indefinite history of influenza, and the hepatic condition was associated in its beginning with cough and hypochondriac pain. At autopsy the origin of the infection was not found, but from the likeness of the hepatic abscess to those seen in earlier cases, a portal type of distribution of the infection seems most probable. The multiple abscesses of other organs found at autopsy probably represented pyemic dissemination of infection from the old hepatic focus. The long clinical course, with persistence of a draining sinus, suggested actinomycosis. Anatomically the similarity was also marked (Fig. 4).

CASE 8. The illness of a man, 39 years of age, began in February, 1924, with persistent abdominal pain, fever and diarrhea. These severe symptoms were present for three weeks, but the abdominal pain persisted for four months. After it had subsided, weakness, anorexia and occasional attacks of pain continued. The illness continued with alternating periods of well-being and of exacerbation until the patient's death November 5, 1926, about two years and nine months after the first symptoms.

At autopsy the liver weighed 4250 gm. There were dense, fibrous adhesions between the right lobe and the inferior surface of the diaphragm. A fluctuating mass appeared in the right lobe, which on section was shown to be an abscess. It was 12 cm. in diameter and contained about 1000 cc. of thick, greenish yellow pus that was not bile-stained. Its capsule was from 0.5 to 1 cm. thick and appeared to be composed of white fibrous tissue clearly demarcated from the surrounding, compressed hepatic substance. There were 2000 cc. of ascitic fluid. Chronic and healed ulcers of the ascending colon also were seen. Evidence of pylephlebitis was not found.

Microscopically, the capsule of the abscess consisted of old, fibrous connective tissue, which in its outer part contained bile ducts and the vascular structures of the liver, and sometimes atrophic clusters of hepatic cells. As the cavity of the abscess was approached the connective tissue appeared younger, until fibroblasts and endothelial sprouts of blood capillaries composed the microscopic fields. In this younger connective tissue there was dense leukocytic infiltration; predominating types were lymphocytes, large mononuclear leukocytes and plasma cells. A layer of fibrin surrounded the abscess cavity in which fibroblasts were developing. In this zone Gram-positive micrococci in short chains were seen. In the exudate

citis, but attention was never focused on the liver until fully nine months had elapsed. During this time, the progressive chronic empyema, which persisted in draining, seemed to account for all the symptoms. As this cleared, however, the evidence of hepatic supuration was revealed. Whether the appendix was the primary focus of infection is not clear, but that the abscess was of portal embolic origin seems certain. The empyema was probably secondary to the hepatic involvement, but the thought that the abscess of the liver may have followed the empyema, with involvement of the right hepatic lobe, should at least receive some consideration. The only evidence that the appendix was the primary focus consisted in the finding of an adherent tip, with obliteration of the lumen of the adherent portion.

CASE 7. In April, 1926, a woman, 19 years of age, noted the onset of cough, which persisted with mild attacks of pain in the right hypochondrium. In June, abdominal exploration revealed an abscess in the right lobe of the liver. Drainage was instituted and persisted up to the time of her death. Actinomycosis and tuberculosis were repeatedly excluded by study of the material which drained. As a terminal feature there were multiple subcutaneous abscesses, and osteomyelitis of the right tibia. The patient died in January, 1928, about one year and nine months after symptoms referable to the liver appeared.

At autopsy the liver weighed 1800 gm. A large, multilocular abscess 7 cm. in diameter was found in the right lobe of the liver. The border was sharply demarcated from the surrounding liver, but a heavy fibrous encapsulating membrane was not present. The surrounding hepatic tissue was compressed and congested. The large abscess appeared to be composed of multiple confluent small abscesses, each surrounded by an irregular zone of grayish white, firm substance in which yellowish foci were visible. Small abscesses were found in the lungs, pancreas, spleen, kidneys, ankles, elbows and wrists. There was no evidence of pyelephlebitis.

Microscopically the encapsulating membranes of each suppurating focus of the multilocular hepatic abscess were of compact connective tissue in the peripheral part, which changed to a fibroblastic type of tissue toward the zone of suppuration. Here, too, numerous endothelioid cells were crowded together. Giant cells of foreign body and Langhans' type appeared in this zone. The periphery of the exudate was composed of polymorphonuclear leukocytes, but many mononuclear leukocytes were interspersed. Centrally the exudate took the eosin stain and was of acellular, hyaline appearance. Colonies of bacteria were sparse, Gram-Weigert stained sections revealing only a few staphylococci. No actinomycetes or *Mycobacterium tuberculosis* were found.

Other cases in which the periods of illness have been shorter have been reviewed in an effort to show the transitional stages between the early and the late cases. These cases present similar characteristics, but are representative of varying degrees of chronicity, and show that the extremely latent stages are but a continuation of the reactions which may exist to some degree in earlier cases.

In only one of my cases was the source of the hepatic lesion without question. In Case 2 there is little doubt that the perforated duodenal ulcer induced the infection from which the hepatic suppuration arose. In three cases, appendicitis was to some extent suggested by the clinical history as having been the starting point for the hepatic abscess. In Case 5 the appendix had been removed five years before onset of the hepatic abscess. In this interval the patient's health was good, and to suppose that a pyogenic focus had persisted for five years, from which the hepatic lesion developed, carries one too far into the speculative field. In Cases 4 and 6 the clinical evidence at first pointed to the appendix, but at autopsy the appendix was found to be but slightly altered. Some evidence existed in both cases of previous disturbance in the appendix, but with healing completed it was impossible to prove beyond question, from pathological studies, that the appendix had been the primary focus of infection. In Cases 5 and 8 there were non-specific ulcerative lesions in the bowel; these could have constituted the focus from which infection of the liver had developed. In Case 6 the question of a primary appendiceal focus with secondary empyema of the right side and extension from that to the liver, appeared to be the clinical course of the illness. It is more reasonable, however, to believe that portal infection was masked by the empyema, and that the empyema was in reality an hepatic complication, by extension of infection through the diaphragm. The evidence of hepatic involvement became apparent as the empyema subsided. The source of the infection in Case 3 presented difficulty in interpretation. There seemed to be a relation between the cervical suppuration and the hepatic abscess, in that one preceded the other. The hepatic lesion, however, had almost unmistakable characteristics of an abscess of portal origin. It is possible and reasonable to assume that secondary foci were established in the lungs at the onset of the final severe illness, when cough was the principal complaint. Secondary distribution of infection could have occurred within the field of portal drainage and a third

itself considerable necrosis was apparent, but polymorphonuclear leukocytes still persisted.

Gram-Weigert stains of the exudate and of the tissue revealed Gram-positive diplococci, often in short chains.

*Comment:* The remarkable feature of this hepatic abscess was found in its persistence for so long a time with so little clinical evidence of its presence. Its origin is uncertain. At the onset, abdominal pain, fever, and diarrhea were the only clinical signs, and careful attention then eliminated any specific disease which would account for these manifestations. In its solitary character (Fig. 5) it resembled abscesses of amebic origin, but this could not be proved by examination, either of the abscess or of the intestinal flora. The chronic and healed ulcers of the ascending colon had no specific characteristics by which they could be identified, but the possibility of their being residual from the primary infection had to be considered.

## DISCUSSION

From this study it may be concluded that in certain instances, either due to diminished or specific virulence of the microorganisms, or to the specific resistance of the host, pyogenic micrococci may involve the liver in abscess formation which may persist into a chronic granulomatous stage. This characteristic is displayed by these microorganisms in other anatomical situations. There are, for instance, the well known pyogenic granulomas of cutaneous tissues, and the chronic infections of bones in which pyogenic cocci are the causal microorganisms. As far as the liver is concerned, abscesses of long duration, such as those due to ameba, frequently have been emphasized. Attention has been directed previously to the persistence of pyogenic abscesses until they advance into a chronic stage, and also to their insidious progression. That hepatic abscesses, in which the pyogenic cocci are specific etiological agents, induce typical granulomatous changes in the liver such as those which develop in actinomycosis, tuberculosis and blastomycosis, to my knowledge has not been emphasized hitherto. In this series I have reported three cases in which the advance into chronicity was most insidious. These have been of duration, respectively, ten months (Case 6), one year and nine months (Case 7), and two years and nine months (Case 8).



having their formation on the earlier, purely hepatic basis by the recognition of biliary duct structures which persist in them.

I have seen and described two types of pyogenic abscess which may persist into the extremely chronic stages; the one is a solitary cavity (Case 8, Fig. 5) resembling the solitary amebic abscess, and the other the multilocular or honeycomb abscess resembling the lesion of actinomycosis (Cases 6 and 7, Fig. 4). The granulomatous characteristics of each type are similarly manifested in studies on their capsule, but the reaction to formation of solid granulomatous portions, replacing smaller multilocular abscess cavities, was shown only by the latter type. The microscopic characteristics of the pyogenic granuloma in general reveal those features which are grossly evident. There is a fibrous encapsulation which, according to duration, exhibits more old or more young connective tissue. The connective tissue reaction, however, appears to be slowly progressive toward resolution of the abscess. Thus fibroblasts, endothelioid cells, large mononuclear leukocytes, lymphocytes and plasma cells permeate the regions of productive reaction, with giant cells even constituting a part of the picture (Fig. 8). The exudate itself, at first predominatingly of polymorphonuclear neutrophilic leukocytes, becomes gradually transformed into partially necrotic detritus in which many mononuclear types of leukocytes appear. Colonies of micrococci appear abundantly in the earlier cases, but later they are identified only with difficulty. When abundant, either the staphylococci or the streptococci may assume in their colonization features like the actinomycetes (Fig. 9); they are easily distinguished, however, by higher magnifications and bacterial stains. That these abscesses represent regressing actinomycosis with disappearance of the actinomycetes is barely possible, but in my experience with the disease, such an eventuality does not occur when organic actinomycosis once becomes instituted. Yeasts, fungi, *Mycobacterium tuberculosis*, and so forth have been considered, but a causal relationship for these organisms never has been demonstrated. I have been forced to believe that the demonstrable micrococci are the etiological factors, in spite of the seeming improbability that the pyogenic micrococci could endure in the liver for a sufficient time to produce a specific pyogenic granuloma.

focus could have been established in the liver by way of the portal vein. This circuitous route seems necessary to explain all factors, if the cervical infection is to be considered as the source of the hepatic infection. A retrograde route, from the right auricle to the inferior vena cava and hepatic vein, seems untenable.

The hepatic pyogenic granulomas partake of some of the general characteristics which other abscesses of portal embolic origin possess. Karsner,<sup>11</sup> Kaufmann,<sup>2</sup> Rössle,<sup>3</sup> Schwartz,<sup>12</sup> and others have described the abscesses of portal embolic origin as being multilocular in their formative period. The multilocular construction appears to be due to a shower of infected emboli being transported to the liver and localizing in some of the intrahepatic branches of the portal vein. The size of the emboli determines the point of hepatic lodgment and the number of them apparently determines the extent to which the multiple abscesses will form. In cases unassociated with thrombosis of the main or large branches of the portal vein, the actual emboli may not be found even in microscopic preparations, because the small emboli form the centers about which the abscesses develop, and the emboli lose their identity in the field of suppuration very early. This multilocular characteristic is never found in abscesses in which the method of transportation of bacteria is other than through the portal vein. It is always, however, the outstanding characteristic of the hepatic abscess that is known to be disseminated by emboli that pass through the portal circulation. Examination of the abscesses shortly after their formation will show that the limiting encapsulating membrane of each unit of the multilocular abscess is composed only of hepatic tissue. Ordinarily progressive suppuration should dissolve these partitions, and a single cavity results. The partitions in the granulomatous abscesses, however, usually persist to a greater or less degree, and on them the fibroblastic proliferation and other productive reactions ensue (Figs. 6 and 7). The rapidity with which this organizational change in the capsule progresses, apparently reinforcing the original barrier of hepatic tissue, determines whether the suppuration and destruction of tissue will continue to the formation of large multilocular cavities (Fig. 2), or of a single cavity (Fig. 5), or whether through excessive productive tissue reactions the original appearance of multilocularity will be preserved (Fig. 4). In the later stages the trabecular strands which form the capsules for the multilocular units may be identified as

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## DESCRIPTION OF PLATES

## PLATE 48

- FIG. 1. Case 1. Subacute, multilocular, pyogenic abscesses involving the right lobe of the liver, with suppuration progressing locally to actual cavitation. The primary hepatic focus is central. Peripheral dissemination is secondary. Associated thrombophlebitis. Difficulty in draining such an abscess is apparent.
- FIG. 2. Case 2. Chronic, multilocular, pyogenic abscess of the right lobe of the liver. Large cavities formed by progressive suppuration. Trabecular, fibrotic strands persisting.
- FIG. 3. Case 3. Chronic, leaf-shaped, multilocular, pyogenic abscesses of the right lobe of the liver.

## SUMMARY

1. Abscesses of the liver, originating from within the field of drainage of the portal vein, form a significant clinical and pathological group. These usually take origin from primary intestinal foci through the production of local thrombophlebitis. The hepatic suppuration develops from the passage of infected emboli from the primary foci by way of the portal circulation to the liver.

2. The primary thrombophlebitis may induce thrombosis of the portal vein and pylephlebitis, or this feature of the lesion may be absent.

3. As the cases emphasize, the primary focus may be cryptogenic. From the appearance of the abscess, however, the source may be suspected. A characteristic distribution and type of abscess is produced by each method of hepatic dissemination.

4. The abscesses of portal origin are at first multilocular, due to multiple foci in emboli. Each multilocular abscess usually remains discrete, although they may be multiple. Involvement of the right lobe alone is most common. General hepatic dissemination, in this type, is unusual.

5. The significance of the pyogenic cocci as etiological agents has been emphasized. The illness associated with abscess of the liver may be extremely insidious, and as the primary focus may be cryptogenic, so also the hepatic lesion itself may exhibit this same characteristic.

6. Progression into extreme chronicity may occur, with preservation of the original multilocular arrangement, or a solitary, adequately encapsulated abscess may result.

7. In the cases of extreme chronicity a granulomatous reaction has been found, with the pyogenic cocci demonstrable as the etiological agents. Such cases resemble the granulomas of actinomycosis in their chronicity and in their granulomatous characteristics.

8. Eight cases representative of pyogenic abscesses, possessing granulomatous changes in various stages of their evolution, have been presented.

PLATE 49

FIG. 4. Case 7. Chronic, multilocular, pyogenic abscess of the right lobe of the liver. Scanty suppuration, granulomatous features predominate.

FIG. 5. Case 8. Large chronic, solitary, pyogenic abscess of the right lobe. Dense granulomatous encapsulation.



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Granulomatous Abscess of Liver of Pyogenic Origin

PLATE 50

- FIG. 6. Case 2. Two units of a multilocular abscess with persisting partition of hepatic derivation, supplemented by early granulomatous reaction in which giant cells are formed (from small secondary foci about large primary abscess).  $\times 140$ .
- FIG. 7. Case 6. Granulation tissue with fibroblasts, vascular sprouts, lymphocytes and mononuclear leukocytes forming the wall of a multilocular abscess unit.  $\times 165$ .
- FIG. 8. Case 7. Late granulomatous reaction with extensive proliferation of fibroblasts, endothelioid cells, giant cells and lymphocytic collections, replacing the detritus of polymorphonuclear neutrophilic leukocytes.  $\times 225$ .
- FIG. 9. Case 2. Colony of staphylococci in center of small abscess assuming morphological resemblance to *Actinomyces hominis*.  $\times 350$ .



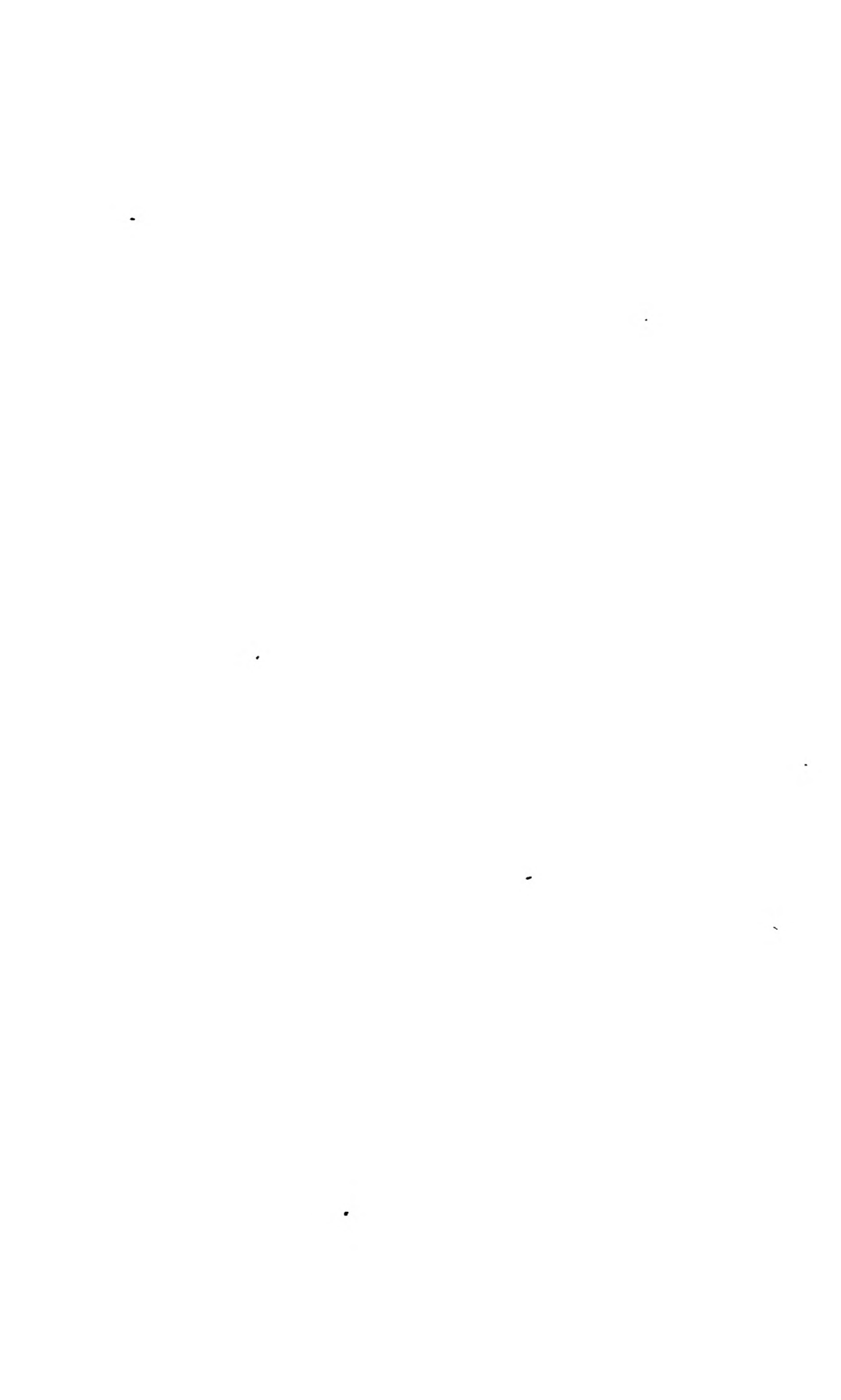
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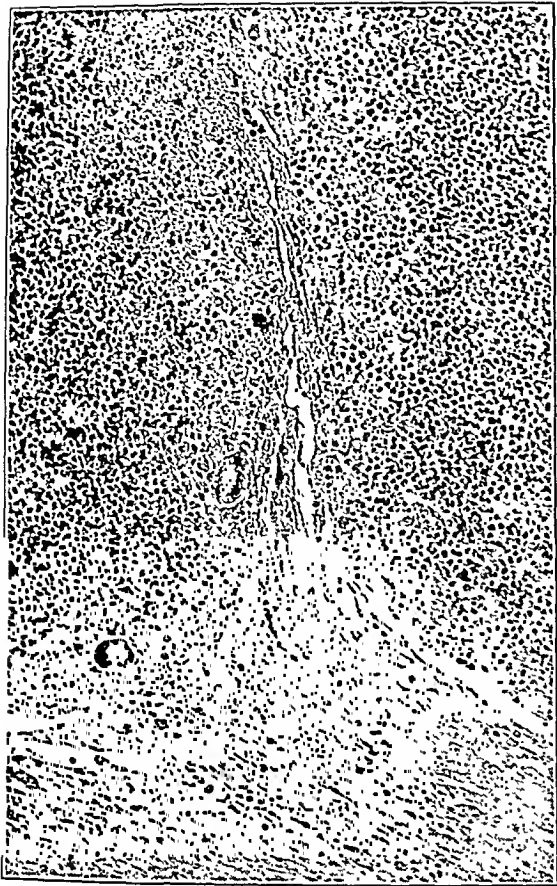


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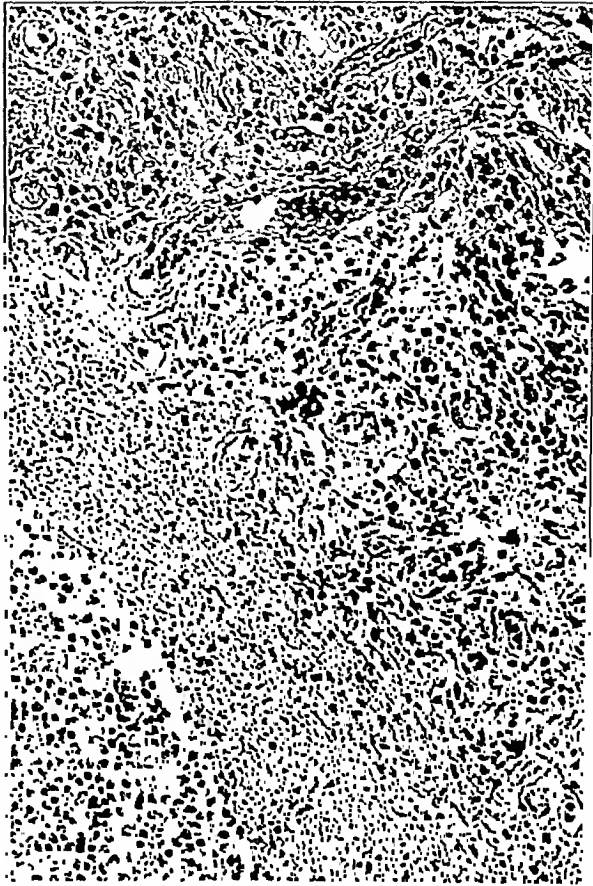
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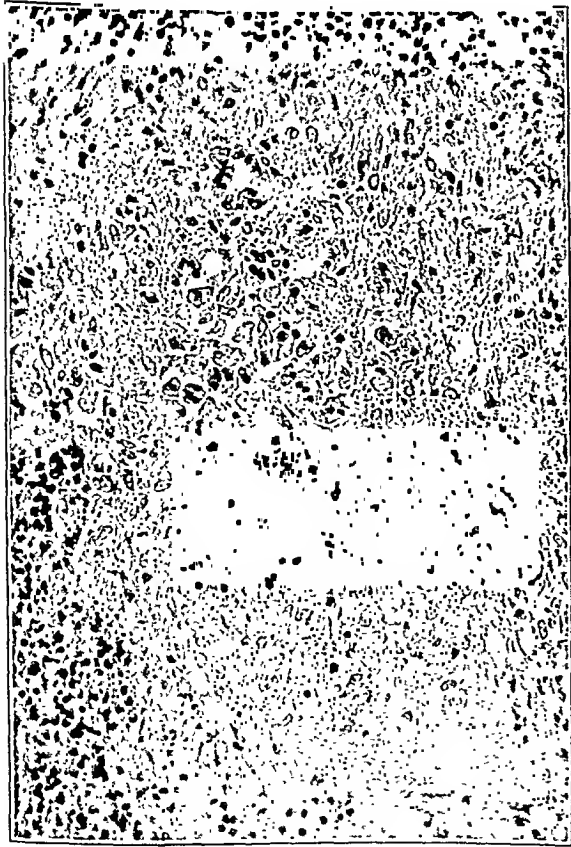




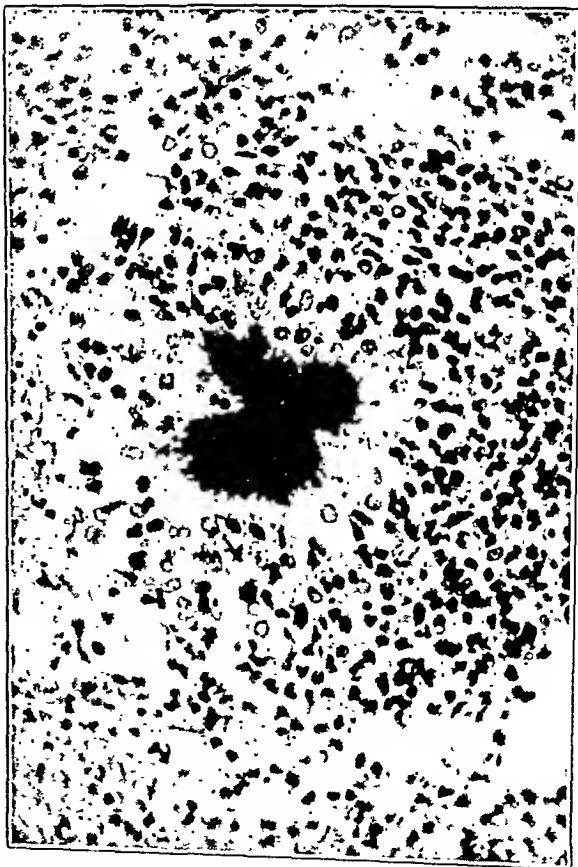
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Granulomatous Abscess of Liver of Pyogenic Origin

ductus arteriosus both patent, the former measuring 5 mm. and the latter 3 mm. in diameter. Interventricular septum intact. Valves and endocardium negative.

*Lungs:* Right lung weighed 46 gm. and was moderately well expanded. The left weighed 38 gm. and was well expanded. Pleural surfaces of both lungs smooth and glistening, but showed scattered petechial hemorrhages measuring up to 2 mm. in diameter. Scattered throughout both lungs, particularly in the dependent portions of both lower lobes, were frequent dark brown hemorrhagic areas which measured up to 2 cm. in diameter. Crepitation reduced in these areas. Slight emphysema noted in the non-dependent portions. A large amount of frothy, serous yellow fluid could be expressed from all portions of the cut surface by pressure. Bronchial mucosa slightly congested.

*Spleen:* Weighed 48 gm., (normal weight 8 gm.). Grayish purple in color and speckled with diffuse gray areas. External surface showed much slight puckering but no inflammatory exudate. Thin sections cut easily and held their shape well. Cut surface a rich reddish brown in color and firm. Malpighian corpuscles could be distinguished but were not enlarged.

*Pancreas:* Negative.

*Gastro-Intestinal Tract:* Mucosa of the stomach moderately congested. Small and large intestines contained a very small amount of meconium.

*Liver:* Weighed 190 gm., (normal weight 78 gm.). Capsule smooth, glistening and not thickened. Liver dark uniform purplish brown in color. Consistence appeared to be normal. No evidence of fatty infiltration or biliary stasis.

*Gall-Bladder:* Negative.

*Adrenals:* Together weighed 10 gm. Negative.

*Kidneys:* The right kidney weighed 19 gm. and the left 19 gm., (normal weight of right kidney 13 gm., left 14 gm.). Cortex measured 2 mm. in thickness. Negative except for fetal lobulations and reddish brown uric acid deposits in medulla.

*Bladder:* Showed several small submucous hemorrhages measuring up to 0.5 mm. in diameter.

*Genitalia:* Normal in size and development for this age period. Both testicles descended.

*Aorta:* Elastic, and the whole intimal surface markedly icteric.

*Organs of the Neck:* Trachea, thyroid, submaxillary glands and a small portion of jaw removed. These structures were negative.

*Bone Marrow:* Sections were taken from the ribs and vertebrae. Marrow reddish brown in color, indicating active hematopoiesis. Costochondral junction slightly irregular in outline, but sharp and well defined.

*Spinal Cord:* Sectioned transversely at intervals of 1 to 2 cm. Negative throughout.

*Brain:* Weighed 368 gm., (normal weight 335 gm.). Diffuse icteric tinge and a few petechial hemorrhages into the fossa interpeduncularis. Anterior fontanelle measured 3 by 6 cm. in diameter. No posterior fontanelle seen. Meninges negative. Arachnoid fluid present in normal amount and clear. There was no pressure cone. Brain uniformly firm after fixation in formalin. Coronal sections were made at intervals of 1 to 2 cm. The substance of the brain, particularly near the base and near the ventricles, showed moderate jaundice.

*Bacteriology:* Pneumococcus recovered from the peritoneal cavity.

## ERYTHROBLASTOSIS WITH JAUNDICE AND EDEMA IN THE NEWLY BORN \*

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The object of this report is to place on record a histopathological study of six cases of erythroblastosis in which the etiology was obscure and the pathological findings difficult to interpret in the light of present knowledge.

### CASE I. ERYTHROBLASTOSIS WITH JAUNDICE

*Clinical Note:* Male infant born at 2 P.M. Nov. 3, 1930, after a normal labor of ten hours' duration. Baby breathed and cried well, took warm water well, but showed a moderate degree of jaundice. On the second day he began to vomit yellowish brown fluid. There was no cyanosis and no convulsions. On the third day he vomited yellowish fluid, and later bright red blood. The urine was blood-stained. Marked dyspnea was present beginning on the second day. Death occurred sixty-two hours after birth. Mother and father living and well. This was the fourth child. Three siblings living and well. There was no history of tuberculosis, cancer or lues.

*Postmortem Examination:* White male infant 49 cm. in length. Development and nutrition both good. No edema. The skin showed moderate generalized icterus. A little frothy, serous, slightly bloody fluid flowed from the nostrils when pressure was applied to the chest. The sclerae were markedly jaundiced. Pupils regular, round and equal, 3 mm. in diameter. Small excoriations in both inguinal regions, more marked on the left. Subcutaneous fat moderate in amount and icteric. Musculature well developed. All the organs had a moderate icteric tinge. Liver extended 6.5 cm. below the xyphoid. Spleen extended 4 cm. below the costal margin. The dome of the diaphragm came to the level of the fourth rib on the left and the third intercostal space on the right.

*Pleural Cavity:* Negative.

*Mediastinum:* Contained a thymus weighing 8 gm.

*Pericardial Cavity:* Contained a few centimeters of slightly icteric fluid in which a few flakes of a material resembling fibrin were floating. A smear of this was found to be negative. Surfaces smooth, glistening and free from hemorrhage.

*Heart:* Weighed 26 gm., (normal weight 17 gm.). The valve measurements were as follows: tricuspid valve 3.8 cm., pulmonary valve 2.2 cm., mitral valve 3 cm., and aortic valve 2.1 cm. The myocardium of the left ventricle measured 0.6 to 0.7 cm., and the right measured 0.3 cm. On the posterior surface of the heart were a few scattered petechial hemorrhages, none exceeding 2 mm. in diameter. The myocardium was reddish brown in color. Foramen ovale and

\* Received for publication April 1, 1931.

There were numerous Hassall's corpuscles, some of which were unusually large.

*Thyroid:* Only occasional follicles showed intact epithelial cells and the presence of a small amount of colloid. The majority of the acini were devoid of colloid.

*Bone Marrow:* Active hematopoiesis was indicated by the presence of frequent myelocytes, erythroblasts, normoblasts and megakaryocytes. The line of provisional ossification was sharp and well defined and many of the bone trabeculae near it were incompletely ossified. There was slight irregularity of the columns of cartilage cells next to the line of provisional ossification.

*Brain:* Negative.

*Cord:* A few widely scattered macrophages were found in the meninges.

**SUMMARY:** This was an infant who died at the age of 62 hours, with the clinical diagnosis of hemorrhagic disease of the newly born. The main signs had been bleeding from the mouth and nose, and blood-stained urine. Icterus was present. The main postmortem findings can be divided into two groups. There was some evidence of a pneumococcemia as shown by recovery of pneumococcus from the peritoneum, slight bronchopneumonia and multiple abscesses in the kidneys, and in addition there was aspiration of amniotic contents indicative of a certain degree of intra-uterine asphyxia. The second group of findings included icterus, an enlarged spleen and liver, and foci of hematopoiesis in various organs of the body.

## CASE II. ERYTHROBLASTOSIS WITH JAUNDICE

*Clinical Note:* A male child, sixteen hours after a normal birth at full term, developed difficulty in respiration and died suddenly. The total duration of labor was six hours and nothing untoward was noticed at any time. The placenta appeared normal. There were two other children alive and well. The mother's history was entirely negative.

*Postmortem Examination:* Body was that of a well developed, decidedly icteric male infant, weighing  $7\frac{1}{2}$  pounds. Slight edema of the scrotum. Finger nails bluish in color. Umbilical cord showed no evidence of hemorrhage or infection. Fontanelles normal. A slight, reddish, frothy discharge was present around the nose. Subcutaneous tissues moist. The fatty layer measured 0.5 cm. in thickness and was markedly icteric.

*Peritoneal Cavity:* Negative.

*Pleural Cavities:* Negative.

## MICROSCOPIC EXAMINATION

*Heart:* Essentially negative.

*Lung:* Showed many small hemorrhages, chiefly intra-alveolar. A few of the extravasated red blood cells were nucleated. A few alveoli contained polymorphonuclear leucocytes and macrophages. Others showed bits of vernix and a few cornified epithelial cells. The interlobular connective tissue septa were edematous and infiltrated by a few macrophages. There was moderate postmortem desquamation of bronchial epithelium. Many bronchial lumina contained red blood cells.

*Spleen:* Moderate congestion was present and there was marked hematopoiesis in both the erythroblastic and myelocytic series, with the former predominating. A moderate number of pigment-laden macrophages were observed. There was no hemorrhage, necrosis or increase in connective tissue and no acute inflammatory cell infiltration. Follicles were not hyperplastic. The section stained by Levaditi's method showed no spirochetes.

*Pancreas:* Pancreatic interstitial connective tissue was slightly but definitely increased and somewhat edematous. It showed very slight hematopoiesis.

*Gastro-Intestinal Tract:* Negative.

*Liver:* The outstanding feature was marked embryonic hematopoiesis in which both myelocytes and normoblasts were represented, with the latter greatly predominating. The liver cells were well preserved, but showed a number of small fatty vacuoles. The bile capillaries were not dilated and the bile ducts were well preserved. No spirochetes were found.

*Adrenals:* Numerous foci of hematopoiesis were seen in various parts of the sections.

*Kidney:* In the medulla of both kidneys next to the pelvis were several small to moderate sized discrete and confluent abscesses with necrosis. Hemorrhages into the abscess had also taken place. Moderate generalized congestion was present, particularly in the medulla, and the vessels contained many nucleated red blood cells and frequent myelocytes. A few tubules contained uric acid crystals. The glomeruli were normally formed.

*Thymus:* Showed moderate hematopoiesis in the edematous interlobular stroma, and slight hematopoiesis in the thymic parenchyma.

measuring about one micron or less. In addition there were numerous smaller granules which were stained dark blue. The cytoplasm of the cells was stained a very light blue and contained no granules. Surrounding these nodules were many cells which appeared to be erythroblasts, and around the periphery were a few erythrocytes. Myelocytes and occasional myeloblasts were observed between the liver cords which in many places seemed to be compressed. The liver cells and bile capillaries contained considerable masses of yellowish bile pigment.

*Adrenals:* Showed nodules of hematopoietic tissue.

*Kidneys:* The cortex and medulla contained an occasional focus of blood-forming cells.

*Thyroid:* Foci of erythroblastic tissue were found in the thyroid. The follicles for the most part were not fully developed. A very slight amount of colloid was present.

*Bone Marrow:* Hyperplastic. Primitive blood-forming cells, erythroblasts and myeloblasts predominated. Numerous megakaryocytes were observed also. Very few erythrocytes were found. The stroma of the marrow was very delicate.

*Central Nervous System:* Sections through meninges and choroid plexus showed small foci of hematopoiesis.

*Special Stains:* Several sections from heart muscle and liver were made and stained by Levaditi's method with negative results.

*Bacteriology:* Smears stained by the Gram-Weigert method, and cultures from the lungs, spleen, heart's blood and peritoneal cavity were negative.

**SUMMARY:** In this case there was marked jaundice with bile stasis in the liver, enlargement of the liver and spleen, slight bleeding into the pulmonary alveoli, and abnormal hematopoiesis in the liver and other organs. There was no abnormality of the biliary tree.

### CASE III. ERYTHROBLASTOSIS WITH JAUNDICE

*Clinical Note:* A well developed female child, after a normal delivery at full term, developed difficulty in respiration and died twenty-four hours later. In view of a slight bloody discharge from the mouth and rectum before death, transfusions were attempted. The mother's history was entirely negative.

*Postmortem Examination:* Body markedly jaundiced. No gross anatomical malformations observed. Abdomen markedly enlarged. Subcutaneous fat thin, measuring about 2 mm., and decidedly icteric. No excess fluid or blood found in the peritoneal, pericardial or pleural cavities.

*Heart:* Negative.

*Heart:* Weighed 35 gm. Foramen ovale patent and measured 1.2 cm. in diameter. Heart muscle dark reddish in color and fairly firm. Valves and endocardium negative.

*Lungs:* Left weighed 48 gm. and was collapsed. Cut surface showed a patchy congestion in both upper and lower lobes. The bronchi contained a slight amount of reddish frothy material. Right lung weighed 46 gm. and was similar to left.

*Spleen:* Weighed 47 gm. Capsule smooth and on section cut surface dark red in color and firm in consistence. Malpighian corpuscles not evident.

*Pancreas:* Weighed 7.5 gm. Slightly reddish in color and fairly firm.

*Gastro-Intestinal Tract:* The large intestine contained meconium.

*Liver:* Weighed 208 gm. Right border extended to the true pelvis. Uncut surface as well as cut surface dark red in color and very firm. Gall-bladder was negative.

*Adrenals:* Combined weight of adrenals 6.5 gm. No evidence of hemorrhage.

*Kidneys:* Capsule thin. Surface of kidneys red and smooth on section and structure stood out clearly. Right kidney weighed 15 gm. and left 17 gm.

*Bladder:* Negative.

*Organs of the Neck:* The thymus weighed 4 gm., reddish gray in color and firm. Thyroid grayish red in color.

*Bone Marrow:* Red and soft. Femur, vertebra and ribs examined. Epiphyseal line at the upper end of femur well defined.

*Spinal Cord:* Negative.

*Brain:* Weighed 275 gm. Negative.

## MICROSCOPIC EXAMINATION

*Heart:* Negative.

*Lung:* The alveoli were filled with red blood cells and serum precipitate in many places. Many of the red blood cells were nucleated. A few areas showed a slight emphysema. There was a slight exudate in the bronchi in places, which appeared like a colloid material. The lymph nodes at the hilum showed hematopoiesis.

*Spleen:* The malpighian bodies were small. The sinusoids and the pulp were congested with red blood cells. Many areas of hematopoiesis were observed, also an occasional mononuclear cell containing hemosiderin.

*Pancreas:* A few erythroblasts were found in the interlobular septa.

*Gastro-Intestinal Tract:* Section showed a few small foci of hematopoiesis in the submucosa.

*Liver:* Showed a great many foci of active hematopoiesis with embryonic blood-forming cells. The nuclei of these cells stained a light blue with eosin-methylene blue. They measured about five microns in diameter and contained two to three chromatin masses



*Special Stains:* Seven Levaditi preparations from the liver were negative for spirochetes.

**SUMMARY:** The findings in this case were abnormal hematopoiesis, icterus, partial atelectasis with slight aspiration of vernix and slight bleeding from mouth and rectum.

#### CASE IV. ERYTHROBLASTOSIS WITH NEITHER JAUNDICE NOR EDEMA

*Clinical Note:* An infant twelve hours after normal birth at a time estimated to be in the latter part of the ninth month, developed difficulty in respiration and died. The infant was reported to be passing bloody urine prior to death. The mother's history was negative. There was one other child, 2 years old, alive and well.

*Postmortem Examination:* Body showed no gross anatomical lesion of any nature except an enlarged abdomen. The layer of subcutaneous fat was practically absent.

*Heart:* Muscle firm and reddish brown in color.

*Lungs:* Atelectasis observed toward the base. Small portion sank in water.

*Spleen:* Weighed 90 gm. Capsule smooth and reddish brown in color. On section cut surface firm and uniformly dark red in color.

*Pancreas:* Normal in size and consistence.

*Liver:* Weighed 280 gm. Left lobe nearly as large as right. Surface smooth. Capsule not thickened and on section cut surface firm and reddish brown in color.

*Gall-Bladder:* Negative.

*Adrenals:* Negative.

*Kidneys:* Negative.

*Bladder and Ureter:* The bladder was connected to the region of the umbilicus by the urachus. On section about 1 cc. of bloody fluid observed. Ureters appeared normal.

#### MICROSCOPIC EXAMINATION

*Heart:* Heart muscle appeared normal.

*Lung:* Showed atelectasis. In areas where the lung had expanded some of the alveoli were filled with a homogeneous pinkish-staining fluid. The bronchi showed slight desquamation of epithelium.

*Spleen:* There were numerous nucleated red blood cells, erythroblasts and myeloblasts in the pulp. The splenic lymph nodules did not stand out clearly.

*Pancreas:* Appeared normal. Foci of hematopoiesis were observed in the connective tissue around the pancreas and in the connective tissue between the lobes.

*Lungs:* Lungs appeared normal on surface. On section small foci were observed which suggested atelectasis, and some areas were slightly hemorrhagic.

*Spleen:* Just visible beneath left lobe of liver and weighed 55 gm. Capsule smooth, and on section cut surface firm and uniformly reddish brown in color.

*Gastro-Intestinal Tract:* Lower portion of colon contained meconium.

*Liver:* Weighed 300 gm. and extended 7 cm. below costal margin on the right side in mid-axillary line. Surface firm and dark red in color. Cut surface very firm and dark red in color.

*Gall-Bladder:* Negative.

*Adrenals:* Appeared normal in size.

*Kidneys:* Negative.

*Thymus:* Appeared slightly enlarged and was covered with a blood clot due to the injection of blood.

### MICROSCOPIC EXAMINATION

*Lung:* Showed considerable atelectasis. The bronchi in places contained a hyaline-like material which enclosed cornified cells.

*Spleen:* The spleen showed many dilated capillary spaces which contained numerous blood cells, many of which were hemolyzed. The pulp contained many nucleated red blood cells. In many areas there was considerable hemosiderin, partially free in the pulp and partly in mononuclear cells. The follicles were small but apparently normal in number.

*Liver:* Showed a marked hematopoiesis. The foci, which were numerous, appeared to be producing various types of red blood cells (erythroblasts and normoblasts). The periportal connective tissue showed hematopoietic activity and numerous myelocytes and myeloblasts were observed. The cytoplasm of the liver cells was very faintly staining and in places in the liver cords and even in the liver cells needle-like brownish masses of bile pigment were observed. The sinusoids were dilated at the expense of the liver cords. A large number of hematopoietic foci were located in the liver cords. Mitotic figures were observed in the erythroblasts. In many places the liver cells appeared to be loaded with fat.

*Adrenals:* A few foci of hematopoiesis were observed in the cortex.

*Kidney:* In some areas the cells of the proximal convoluted tubules showed a fine granulation with small vacuoles in the cytoplasm. Foci of hematopoiesis were observed in the kidney and in the wall of the pelvis.

*Thymus:* Showed active hematopoiesis.

*Lungs:* Collapsed, and filled approximately half the chest cavity. Firm and rubbery in consistence, dark reddish purple in color.

*Spleen:* Weighed 22 gm. Firm in consistence and dark red in color.

*Pancreas:* Negative.

*Gastro-Intestinal Tract:* Stomach contained a mucinous material. Lower part of small intestine contained a considerable amount of fluid and greenish black meconium.

*Liver:* Firm and weighed 150 gm. Cut surface rusty brown and lobules could not be made out.

*Gall-Bladder:* Negative.

*Adrenals:* Negative.

*Kidneys:* Negative.

*Bladder:* Negative.

*Uterus, Tubes and Ovaries:* Negative.

*Aorta:* Negative.

*Organs of the Neck:* Thyroid very small, each lobe being about 8 mm. in its greatest dimension.

*Bone Marrow:* Bright red color in ribs and vertebra.

*Spinal Cord:* Appeared entirely negative in lumbar region.

*Brain:* Beneath the scalp was a collection of clotted blood and serum about 1 cm. in depth. Sutures almost completely closed. Posterior fontanelle completely closed, while the anterior fontanelle measured 1.5 cm. in its greatest length, and 8 mm. in its greatest diameter. Cerebral venous sinuses distended with clotted blood.

## MICROSCOPIC EXAMINATION

*Heart:* Negative.

*Lung:* The lungs were unexpanded.

*Spleen:* The spleen showed active hematopoiesis. The pulp was congested with cells and contained numerous embryonic cells of the red blood series and numerous normoblasts and erythrocytes. The lymph follicles were small.

*Pancreas:* Showed foci of hematopoiesis.

*Intestine:* The duodenum showed numerous small foci of nucleated red blood cells in the submucosa.

*Liver:* Numerous foci of blood-forming cells were scattered through the liver parenchyma. These foci contained from one to two dozen cells. The nucleus of these cells was slightly larger than an erythrocyte and was composed of a clear staining background in which about a dozen chromatin masses were spread, more particularly around the periphery of the nucleus. The cytoplasm immediately around the nucleus was basophilic in staining reaction and gradually faded in intensity until the border of the cell was indistinct. Mature erythroblasts were present and also numerous normo-

*Liver:* The liver cords contained hematopoietic foci and the nuclei of the liver cells were difficult to distinguish from the embryonic hematogenic cells. Here the liver cells were small and compressed, probably due to the space occupied by the hematopoietic foci, and showed slight fatty degeneration. Numerous eosinophilic myelocytes were observed in the periportal connective tissue along with myeloblasts. In certain areas the liver cells contained fine brownish granules, probably bilirubin. There were great numbers of erythroblasts showing mitoses in these sections.

*Adrenals:* Showed foci of hematopoiesis.

*Kidneys:* In places the tubules appeared to be plugged with nucleated blood cells. The cells of the proximal convoluted tubules showed a marked pyknosis of the nuclei with a pale staining, swollen cytoplasm. Many areas of hematopoiesis were observed throughout the sections.

*Bladder:* A few nucleated red blood cells were observed in the submucosa.

*Thymus:* Showed foci of hematopoiesis.

**SUMMARY:** The findings in this case were enlargement of the liver and spleen, blood-stained urine, slight degeneration of the cells of the proximal convoluted tubules and abnormal hematopoiesis in the liver, spleen and other organs.

## CASE V. ERYTHROBLASTOSIS WITH EDEMA

*Clinical Note:* Female infant died during birth. The exact time the fetal heart stopped beating was not known. Head impacted and presentation transverse. The mother was a multipara in good health and with a negative history.

*Postmortem Examination:* A full term, female white infant, weighing  $7\frac{1}{2}$  pounds. Marked pitting edema of entire body, most marked in the upper part. Hair on the scalp unusually abundant and dark colored. Subcutaneous tissues over the chest and abdomen 8 to 12 mm. in depth. Their thickness was due largely to edema. Pectoral and abdominal muscles greatly swollen, pale yellowish gray in color.

*Peritoneal Cavity:* Contained a large amount of clear straw-colored fluid, estimated to be about 500 cc.

*Pleural Cavities:* Each contained about 150 cc. of clear straw-colored fluid.

*Mediastinum:* Contained a thymus of average dimensions estimated to weigh about 4 gm.

*Pericardial Cavity:* Slight increase in fluid, approximately 25 cc.

*Heart:* In both visceral and parietal layers of pericardium were a few petechial hemorrhages varying from 1 to 1.5 mm. in diameter. Myocardium rather pale but normal in consistence. Endocardium and the heart valves negative.

*Pericardial Cavity:* Slight increase of clear, light straw-colored fluid, about 20 cc.

*Heart:* Muscle somewhat soft in consistence, otherwise negative.

*Lungs:* Collapsed and rubbery. Surface pale reddish brown in color.

*Spleen:* Weighed 25 gm. Quite firm, and on section pulp appeared slightly grayish red and in places somewhat soft.

*Pancreas:* Negative.

*Gastro-Intestinal Tract:* No bleeding into the intestinal tract. Cecum on the left of the midline.

*Liver:* Weighed 170 gm. and filled practically the whole right half of abdominal cavity. Dark red in color and fairly firm. Gall-bladder appeared normal.

*Adrenals:* Negative.

*Kidneys:* Each weighed between 4.5 and 5 gm. Negative.

*Bladder:* Negative.

*Genitalia:* Negative.

*Aorta:* Negative.

*Organs of the Neck:* Thymus weighed 4 gm. and extended to the lower part of the aortic arch. Thyroid small, reddish in color and on section there was no evidence of colloid.

*Bone Marrow:* Ribs and femur dark red. Epiphyseal lines regular and clearly defined.

*Spinal Cord:* Negative in the lumbar region.

*Brain:* Cortex extremely soft. Fontanelles open, the posterior admitting the tip of the little finger, the anterior admitting the tips of the first two fingers.

## MICROSCOPIC EXAMINATION

*Heart:* The vessels contained numerous nucleated red blood cells.

*Lung:* Unexpanded. Occasional nucleated red blood cells were observed in the stroma about the blood vessels and bronchi.

*Spleen:* The capsule was thin, the trabeculae were quite delicate and did not stand out. The malpighian corpuscles were small and the pulp in general was congested with erythrocytes and hematopoietic foci.

*Pancreas:* The stroma between the acini was dotted with hematopoietic foci.

*Gastro-Intestinal Tract:* Occasional groups of nucleated red blood cells were observed in the submucosa.

*Liver:* The liver cells were well differentiated although the outline was not distinct in most areas. The sinusoids and the blood vessels in the portal areas contained numerous erythrocytes and nucleated red blood cells. Very few polymorphonuclear leucocytes were observed. Innumerable small foci of hematopoietic cells with dark staining nuclei containing a dozen or more large chromatin

blasts. The erythroblasts showed a slight acidophilic cytoplasm and the nucleus stained a dark blue.

The capillaries were slightly dilated and contained many red blood cells. The liver contained a great amount of bile pigment which was for the most part in the liver cells. The periportal areas showed many eosinophilic myelocytes.

*Kidneys:* The vessels were congested. Numerous small foci of blood-forming cells were observed in the cortex.

*Adrenals:* Negative.

*Bladder:* Negative.

*Lymph Nodes:* The mesenteric lymph nodes showed active hematopoiesis.

*Thymus:* Showed marked blood formation.

*Thyroid:* Showed marked hematopoiesis. The follicles were not well differentiated and contained a slight amount of colloid.

*Esophagus:* Negative.

*Bone Marrow:* The spinal column, rib and femur showed active hematopoiesis. Great numbers of erythroblasts and immature red blood cells were observed, also myeloblasts, myelocytes and megakaryocytes. Bone formation appeared normal.

*Brain:* The vessels of the cortex showed some congestion.

**SUMMARY:** This is a case in which death occurred at birth. The anatomical findings were a generalized edema, moderate enlargement of the liver and spleen, petechial hemorrhages in the scalp and pericardium, unexpanded lungs and numerous foci of hematopoiesis in the liver and other organs.

## CASE VI. ERYTHROBLASTOSIS WITH EDEMA

*Clinical Note:* A female fetus in the ninth month was delivered dead by Cesarean section. The fetal heart was not heard after birth and the cord was not pulsating. The mother had quite marked hydramnios. Otherwise the history was entirely negative. This was the second confinement; another child, 3 years of age, was alive and well.

*Postmortem Examination:* Body well developed and weighed  $4\frac{3}{4}$  pounds. Total length 40 cm. The striking feature about the body was the edema which was generalized and very marked. Abdomen distended and flat on percussion. Skin appeared normal. There was a considerable growth of dark coarse hair. Scalp edematous, also eyelids and face. Subcutaneous tissue very edematous and measured 1 to 2 cm. in thickness over sternum.

*Peritoneal Cavity:* Contained 200 cc. of clear thin fluid.

*Pleural Cavities:* Each contained about 100 cc. of clear thin fluid.

examined in four cases and in each case there was active hemato-poiesis. Cases I, II and III showed jaundice which was extreme in Case II. In Cases V and VI there was a generalized edema.

From the table it will be noted that these cases have certain features in common with well known clinical conditions of infancy and early childhood. There was slight bleeding from the mouth and rectum in Case III, and blood-stained urine in Case IV. Cases I and V showed many small petechial hemorrhages. These findings suggest hemorrhagic disease. On the other hand, in hemorrhagic disease the bleeding is frequently massive in amount and the organs appear practically normal, while in the condition described here the liver and spleen were greatly enlarged and the bleeding was very slight. Furthermore two cases showed generalized edema, and three showed jaundice. The histological picture of the liver, spleen and other organs separates beyond reasonable doubt this group of cases from hemorrhagic disease of the newly born.

Normally at birth the liver has completely ceased to function as a hematopoietic organ. In congenital syphilis the liver frequently shows hematopoietic activity at birth. This is usually accompanied by a retardation in differentiation of the organs. It is stated by Bullard<sup>1</sup> that the hematopoietic activity compensates for more or less fibrosis of the bone marrow. In the cases described here the bone marrow showed active hematopoiesis, also the lack of clinical or pathological evidence definitely excludes that disease.

The marked enlargement of the liver and spleen, which in Case IV was over eleven times larger than normal for a newly born infant, and the active hematopoiesis in the bone marrow, liver, spleen and other organs strongly suggest that these cases may be related to the "anemias" of infancy and early childhood. Schridde<sup>2</sup> has pointed out the resemblance in cases of hydrops congenitus with marked extramedullary hematopoietic activity to *anaemia pseudo leukemia infantum*. In Cooley's congenital erythroblastic anemia the main anatomical features are the spleen and bone marrow. Quoting directly from Cooley's description,<sup>3</sup> "The greatly enlarged spleen in which the capsule is often thickened, is usually dark red and is tough and tenacious. Signs of perisplenitis are often seen. The spleen is poor in follicles which have been crowded out by the increase of the pulp in which erythropoietic or myeloid areas may be present. Both liver and spleen show in varying degrees an increase

masses were observed. The nucleus was on the average slightly smaller than a mature erythrocyte, about 6 microns. The cytoplasm stained blue and the whole cell averaged about twelve microns in diameter. The foci contained from two to a dozen or more of these cells scattered throughout. Erythroblasts were observed at the periphery of the foci. In portal areas, especially where the vessel wall was thinned out, a thin layer of endothelium was all that separated the lumen from foci of the cells already described. The portal areas contained a large number of myelocytes.

*Adrenal:* Nucleated red blood cells were scattered through the cortex, especially near the surface.

*Kidney:* The smaller vessels were congested. Nucleated red blood cells were observed, especially in the medullary portion, and also occasional small foci of hematopoiesis.

*Bladder:* Negative.

*Thymus:* Showed active hematopoiesis. Many cells of the red blood series were observed. Particularly prominent were the myelocytes. Polymorphonuclear leucocytes and normoblasts were observed in fairly large numbers.

*Thyroid:* There was no colloid. The acini were not fully developed and the stroma contained hematopoietic foci.

*Bone Marrow:* Showed active hematopoiesis. There were many erythroblasts, myelocytes and megalokaryocytes.

*Brain:* The vessels of the choroid plexus were loaded with nucleated red blood cells.

*Special Stains:* Seven sections of heart and of liver were stained by Levaditi's method with negative results.

**SUMMARY:** In this case no developmental anomalies were observed. There was no evident relationship between the hydramnios and the edema. The findings were the generalized edema, extramedullary hematopoiesis and degeneration of the tubular epithelium of the kidney.

## REVIEW OF CASES

In reviewing the pathological findings of the cases reported here one finds that the abnormal extramedullary hematopoiesis was the outstanding feature. This feature was common to all cases and was most strikingly portrayed in the sections of the liver. In each instance the spleen and liver were enlarged. The bone marrow was



in interstitial connective tissue. Some observers have laid weight on the presence of nucleated red cells and myelocytes in the liver. The bone marrow is usually hyperplastic and the cortex thin."

This description is quoted at some length because the group of cases described in this report closely resemble the anemia described above in some respects, yet there are important differences between the two conditions. In Cooley's erythroblastic anemia the enlarged spleen and the hyperplastic bone marrow are the main features, while in the cases described here the enlarged liver showing marked embryonic hematopoiesis is an important feature. The spleen is enlarged and shows active hematopoiesis and the capsule is thin and smooth. There is no increase in interstitial connective tissue in the liver or in the spleen. In three of the cases jaundice was present at birth and in two cases there was a generalized edema at birth. The bone marrow in each group of cases showed active hematopoiesis. Cooley's anemia is a disease chiefly of infancy and early childhood, while the infants described in this report died during birth or shortly after, which suggests the possibility that it is a disease condition in which extra-uterine life is impossible.

In order to rule out the possibility of prematurity entering into this condition, nine cases diagnosed prematurity were selected from the autopsy records of the Children's Hospital and the protocols and sections were studied. In three there was no hematopoietic activity in the liver. Six which showed hematopoietic activity in the liver and in the spleen were compared with the cases described in this report. In these six premature infants there were a few foci of hematopoiesis composed of hemoglobin-containing cells which appeared to be normoblasts with an occasional erythroblast. The difference is very striking when one considers that in premature infants which range from the seventh to the ninth month a slight extramedullary hematopoietic activity is found, while in the cases described here, all at or nearly at full term, showed marked extramedullary hematopoiesis with embryonic cells forming the nucleus of the foci.

#### DISCUSSION

The condition known as hydrops congenitus and characterized by a widespread generalized edema is very rare. It is, however, by no means a new disease, as cases were described in the seventeenth

TABLE I

*Summary of Findings in Six Cases of Erythroblastosis*

Case	Fetal age	Postnatal age	Important clinical features	Liver		Spleen		Microscopic findings
				Gross appearance	Weight (normal weight 78 gm.)	Gross appearance	Weight (normal weight 8 gm.)	
I	Full term	62 hours	Jaundice, infection	En-larged and firm	gm. 190	En-larged and firm	gm. 48	Embryonic hematopoiesis in liver, spleen, pancreas, adrenals, thymus and lymph nodes. Active hematopoiesis in bone marrow
II	Full term	16 hours	Intense jaundice	En-larged and firm	208	En-larged and firm	47	Marked embryonic hematopoiesis in the liver, spleen, pancreas, kidney, adrenal, thymus, thyroid and lymph nodes. Hyperplasia of bone marrow. Capillary bile stasis
III	Full term	24 hours	Moderate jaundice, slight bloody discharge from mouth and rectum	En-larged and firm	300	En-larged and firm	55	Marked embryonic hematopoiesis in the liver, spleen, pancreas, kidney and thymus. No demonstrable bile stasis
IV	Latter part of ninth month	12 hours	No jaundice, no edema, bloody urine	En-larged and firm	280	En-larged and firm	90	Marked embryonic hematopoiesis in the liver, spleen, pancreas and kidney
V	Full term	Died during birth	Marked generalized edema	En-larged and firm	150	En-larged and firm	22	Embryonic hematopoiesis in the liver, spleen, pancreas, kidney, thymus, thyroid and lymph nodes. Bone marrow showed active hematopoiesis
VI	Ninth month	Delivered dead by Cesarean section	Marked generalized edema	En-larged and firm	170	En-larged and firm	25	Marked embryonic hematopoiesis in liver, pancreas, kidney, adrenals, thymus. Active hematopoiesis in bone marrow

The bile stasis in the liver cells and bile capillaries in Case I is an unusual finding. Icterus neonatorum is generally thought to be due to an excessive hemolysis of red blood cells following birth. It is thought by Goldbloom and Gottlieb<sup>20</sup> that "icterus neonatorum is a hemolytic icterus which is the result of post natal readjustment from a condition of oxygen unsaturation to a normal saturation." Smith<sup>21</sup> states that icterus neonatorum appears more often when the blood group of the mother and of the newborn infant does not match. It is well known that there is a marked decrease in the number of red blood cells from birth to about the tenth day of life. Mitchell<sup>22</sup> has observed that infants with jaundice do not show lower average erythrocyte and hemoglobin values than those without jaundice.

In Case II of this report there is undoubtedly bile stasis in the liver cells and bile capillaries. This observation seems to point to an obstruction, regardless of the fact that the circulatory and biliary systems show no gross anomalies. Goldbloom and Gottlieb<sup>23</sup> speak of a hemohepatogenous type of jaundice in the newborn due to an increased destruction of red blood cells, causing the liver to produce an excessive amount of bile. Also there may be an extrahepatic formation which would add to the intensity of the icterus.<sup>24</sup> In Case II there may have been an excessive production of bile, but it is evident that the bile is dammed up in the liver cells and capillaries. Examination of the reticular network by a modified Bielschowsky stain does not show any abnormality of bile duct system. Due to the hematopoietic foci the reticular system appears in a meshwork, whereas normally the bile capillaries appear to run in radiating lines in the liver cords. The relationship of hematopoiesis to bile stasis is unknown. It is observed, however, that the liver cords in places are crowded by the hematopoietic foci, which may produce sufficient pressure to obstruct the finer capillaries.

The most striking feature of these cases and the one common to all is the hematopoietic activity in the liver and other organs. The cytoplasm in the cells which compose the central part of the foci take a blue stain. Sabin<sup>25</sup> states that in chick embryos an azurophilic cytoplasm predominates when hemoglobin stains first. The cytoplasm of these cells stains an even blue, which probably indicates that they are not hemoglobin-containing and are more embryonic than the erythroblasts which show an acidophilic tint to the

and eighteenth centuries. Channing<sup>4</sup> in 1842 reported a case of "dropsy of the foetus" with anemia. Ballantyne<sup>5</sup> in the latter part of the nineteenth century collected about sixty cases from the literature. Some suppose that Hippocrates<sup>6</sup> was speaking of congenital edema when he spoke of the birth of a fleshy fetus — *foetus carnosus*. Many cases have been reported since Ballantyne's series. The more recent include those of Lahm,<sup>7</sup> Oberndorfer,<sup>8</sup> Becker,<sup>9</sup> Gierke,<sup>10</sup> Hueper<sup>11</sup> and others. In spite of all this study, the etiology is still unknown. Many theories have been advanced. Koegel<sup>12</sup> thinks some toxin is the cause of the edema and the proliferation of blood-forming tissues, and suggests lues or nephritis in the mother as a probable cause. Becker thinks the edema is a result of a toxemia of pregnancy. A mechanical basis was thought by Virchow<sup>13</sup> and Osler<sup>14</sup> to be the cause of congenital edema. Recently the mechanical explanation has been put forward by Lahm.<sup>15</sup> Congenital edema may be of mechanical origin, but where no anatomical lesions exist to explain the cause of the edema some other explanation must be sought. Oberndorfer pointed out that in mechanically caused edema there are no changes in the blood-forming tissues.

The appearance of the blood-forming tissues in congenital edema is very interesting. Eichelbaum<sup>16</sup> identified the cells forming hematopoietic foci as erythroblasts and applied the term erythroblastosis to cases of congenital edema with foci of the erythroblasts in the liver and various organs. Erythroblastosis may occur without edema. Pinkerton<sup>17</sup> reported erythroblastosis in a boy, 7½ years of age. He applied this term to a case in which the bone marrow was hyperplastic and the liver, spleen, pancreas, lymph nodes and other organs contained enormous numbers of nucleated red blood cells.

Earlier writers (Sänger,<sup>18</sup> and others) described congenital edema with leukemia. Recently Perez and Jakob<sup>19</sup> reported a case of congenital edema with no anatomical findings which would explain the edema. The blood count was 6,000,000 erythrocytes, 450,000 leucocytes, with numerous eosinophiles, myelocytes and myeloblasts in blood smears. The child lived one-half an hour after birth.

In view of the published data, it appears that the hematopoietic foci may be chiefly erythropoietic and the term "erythroblastosis" applied, or they may be leucopoietic and the term leukemia may be used, or the abnormal hematopoietic picture may occur without the edema.

present in the liver. This may have been due to excessive hemolysis of large numbers of imperfectly differentiated erythrocytes loading the liver cells with bile pigment more rapidly than it could be taken care of by the bile-excreting apparatus. One of the three cases showing jaundice was complicated by an infection, but this was not thought to be related to abnormal hematopoietic activity or to the jaundice. Two cases showed marked edema at birth, which corresponded to the condition known as hydrops congenitus. One case showed neither jaundice nor edema. All cases showed a marked enlargement of the spleen and liver.

2. The features which these cases have in common with well known disease conditions of infancy have been discussed. A few factors which are thought to be of etiological significance have been mentioned. However, the cause is still unknown.

3. The cases described in this report, when considered as a group, are probably representative of a definite disease entity of the newly born, and whatever the etiology may be, the underlying cause is undoubtedly the same in each instance, whether or not the individual case is characterized by jaundice or edema, or whether both jaundice and edema are lacking.

4. The term erythroblastosis in the newly born is applied to the pathological syndrome described in the six cases reported here because it best depicts the anatomical findings.

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cytoplasm. They correspond to the lymphocytoid wandering cell of Maximow<sup>26</sup> or the primitive mononuclear cell of Pappenheim.<sup>27</sup> No explanation can be given for the early embryonic character of the blood formation. Sure, Kik and Walker<sup>28</sup> have shown that young albino rats develop a marked disturbance in hematopoietic activity when suffering from a deficiency of vitamin B. Normally in the bone marrow, the erythroblasts are the youngest cells found and one megaloblast is sufficient to form a focus of erythroblasts.<sup>29</sup> The finding of widespread extramedullary hematopoietic foci which contain cells more embryonic than the erythroblast must be considered quite abnormal.

The selection of a term descriptive of the hematopoietic picture offered some difficulty. Erythroblastosis was used by Eichelbaum to describe the hematopoietic picture in the liver and other organs in hydrops congenitus. Pinkerton used the term in the connection previously stated. The question arises whether the cells of the hematopoietic foci are not more embryonic than the erythroblast. The hemocytoblast of Ferrata is an undifferentiated cell capable of developing blood cells. The early embryonic cells described in the six cases of this report suggest the hemocytoblast. Warren<sup>30</sup> has used the term hemocytoblastoma to describe a malignant tumor of the kidney pelvis with elements simulating bone marrow, and in many places the central cells of the hematopoietic foci in the cases reported here appear similar to the cells of the tumor he described. However, in these six cases the great number of cells which have differentiated to the point of accurate recognition belong to the erythrocyte series. It appears that the tendency of the cells of the hematopoietic foci in the various organs is essentially erythrogenic.

### SUMMARY

1. In the six cases reported, all of which died during birth or shortly after, a detailed description of the pathological findings has been given. The outstanding feature was the occurrence of abnormal extramedullary hematopoietic activity in full term non-syphilitic infants. In each instance there was a persistence of the fetal mode and location of blood-forming activity without a corresponding retardation in general embryological development. Three cases showed marked jaundice at birth. In one of these, bile stasis was

## DESCRIPTION OF PLATES

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### PLATE 51

FIG. 1. Case I. Shows discrete hematopoietic foci and well differentiated liver cells.  $\times 175$ .

FIG. 2. Case II. Liver. Shows the undifferentiated cells of the hematopoietic foci. The bile stasis in the liver cells is evident. Giemsa stain.  $\times 250$ .

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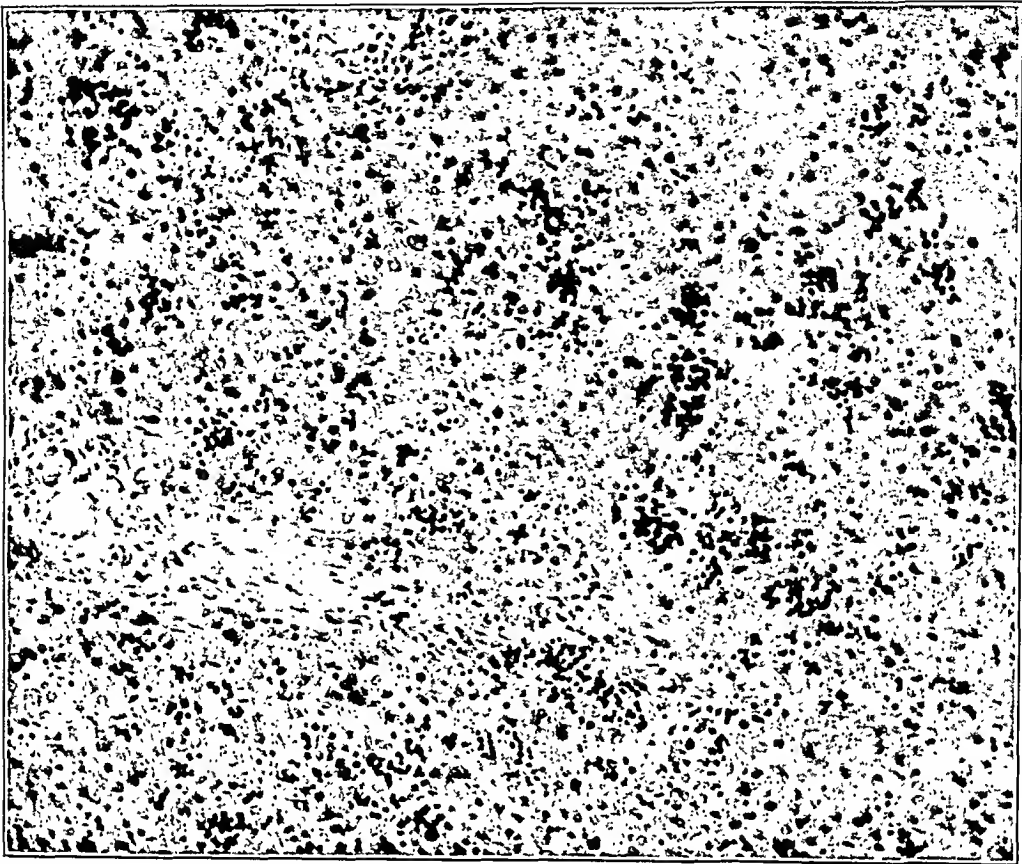
PLATE 52

FIG. 3. Case II. Shows active blood formation in the liver. The liver cells are well differentiated.  $\times 150$ .

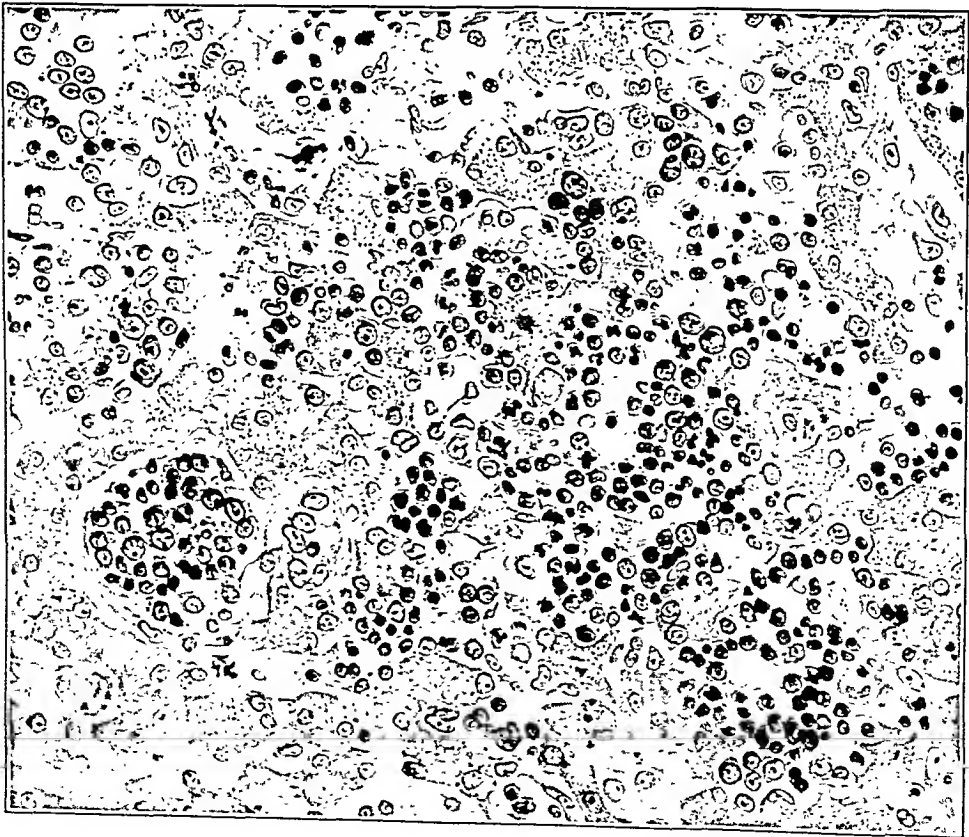
FIG. 4. Case II. One small focus of hematopoietic cells. The difference between the mature erythroblast and the more embryonic red blood cells is shown.  $\times 1000$ .

FIG. 5. Case II. Showing hematopoiesis in the pulp of the spleen and congestion of the sinusoids with nucleated red blood cells.  $\times 750$ .

FIG. 6. Case II. A hematopoietic focus in the kidney.  $\times 500$ .



I

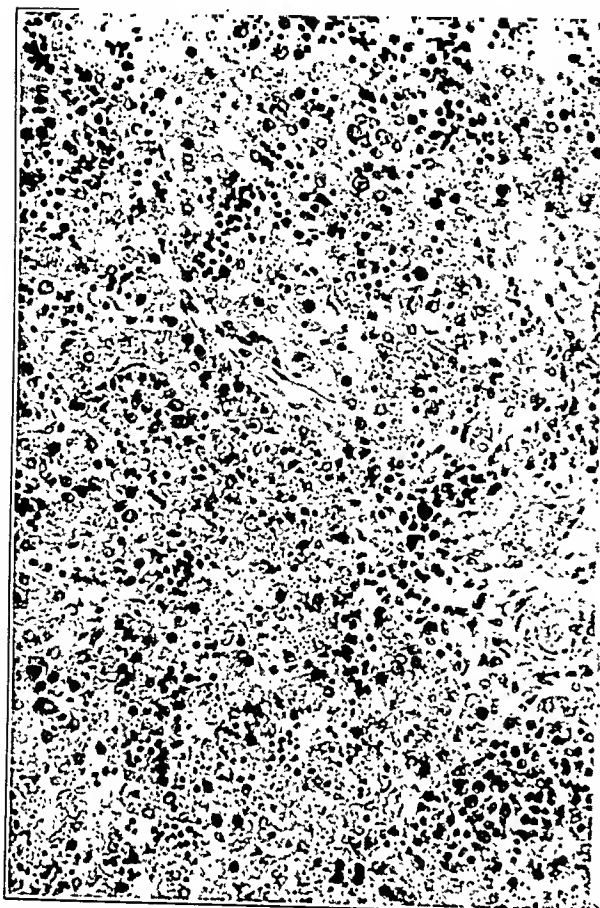


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PLATE 53

FIG. 7. Case IV. Shows many hematopoietic foci in the liver.  $\times 175$ .

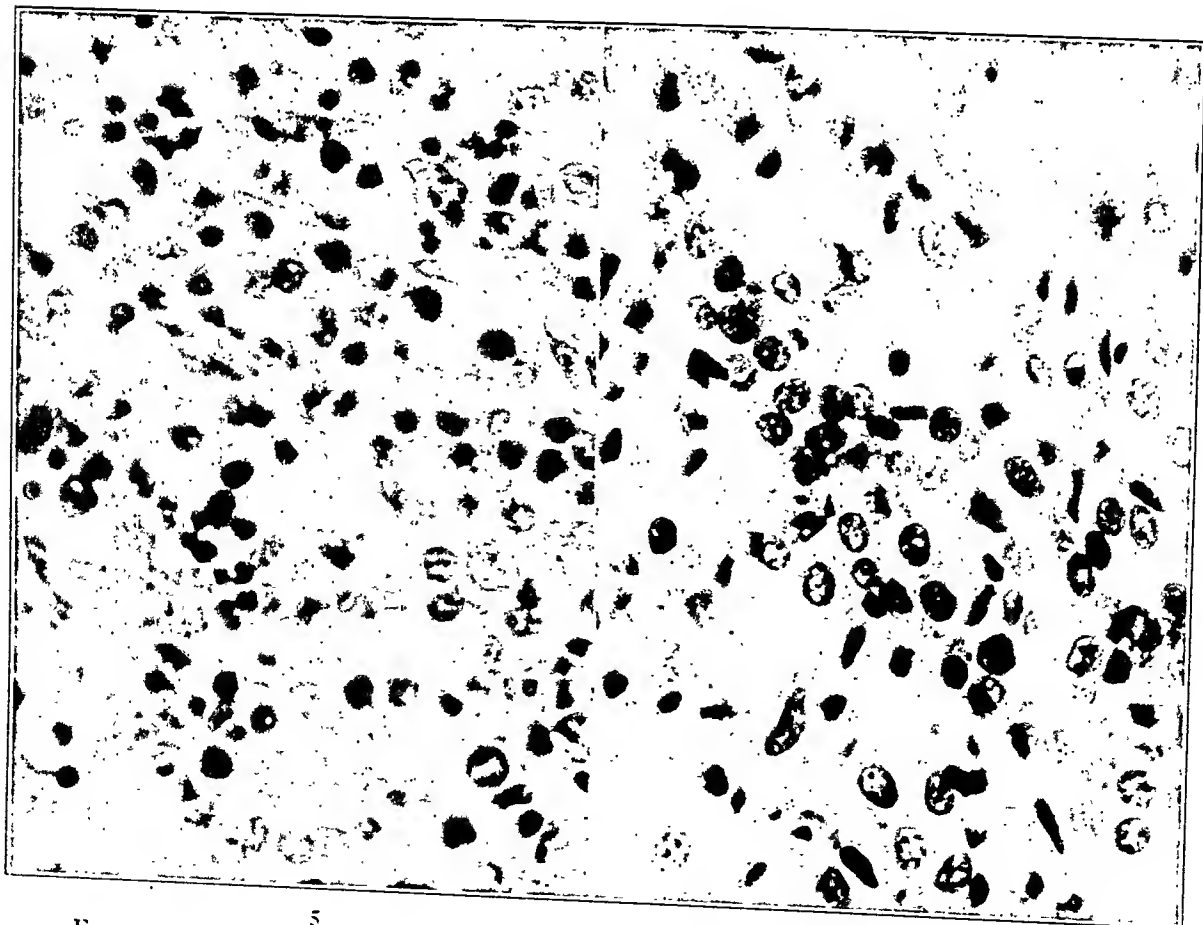
FIG. 8. Case IV. Focus of embryonic hematopoietic cells and erythroblasts in the liver cords.  $\times 1000$ .



3



4



5



6

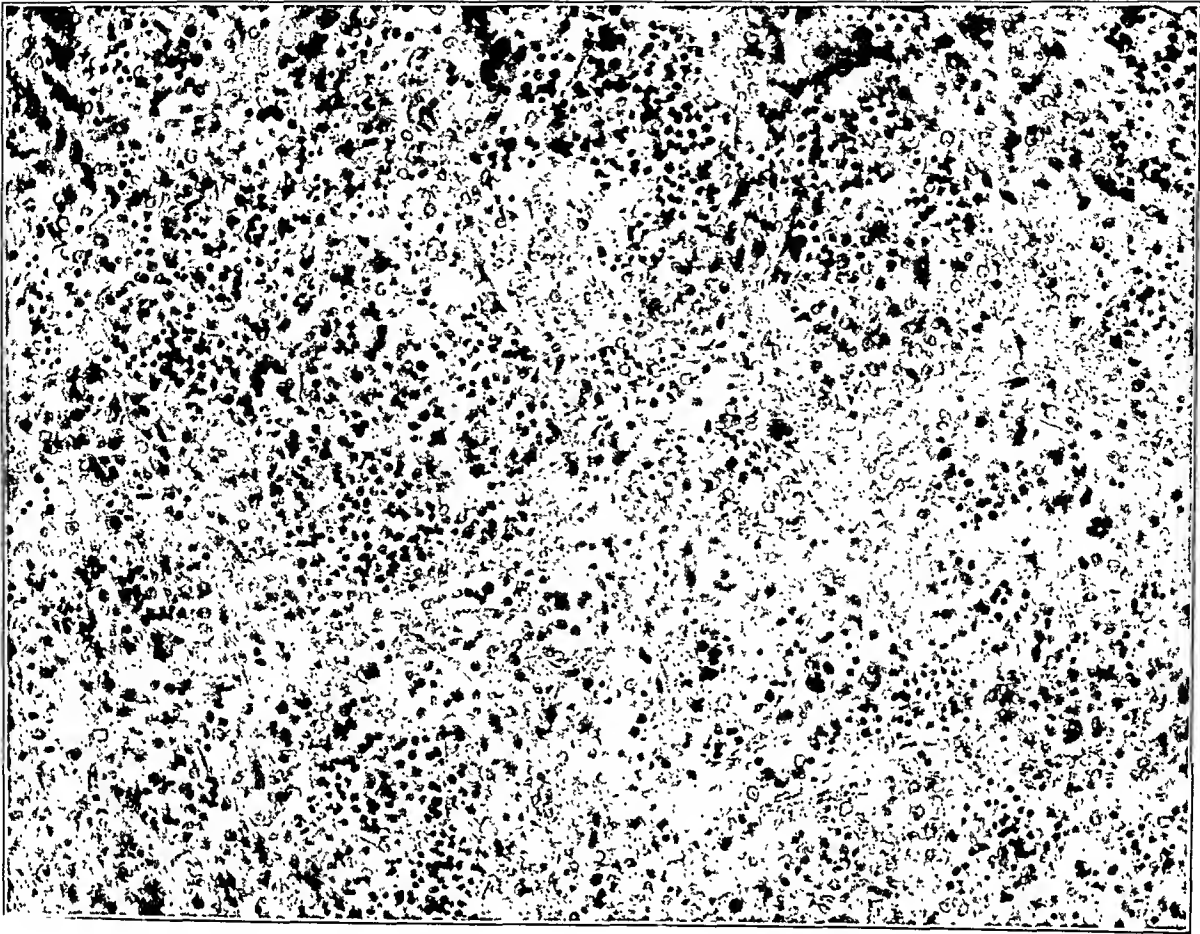
Ferguson

Erythroblastosis in Newly Born

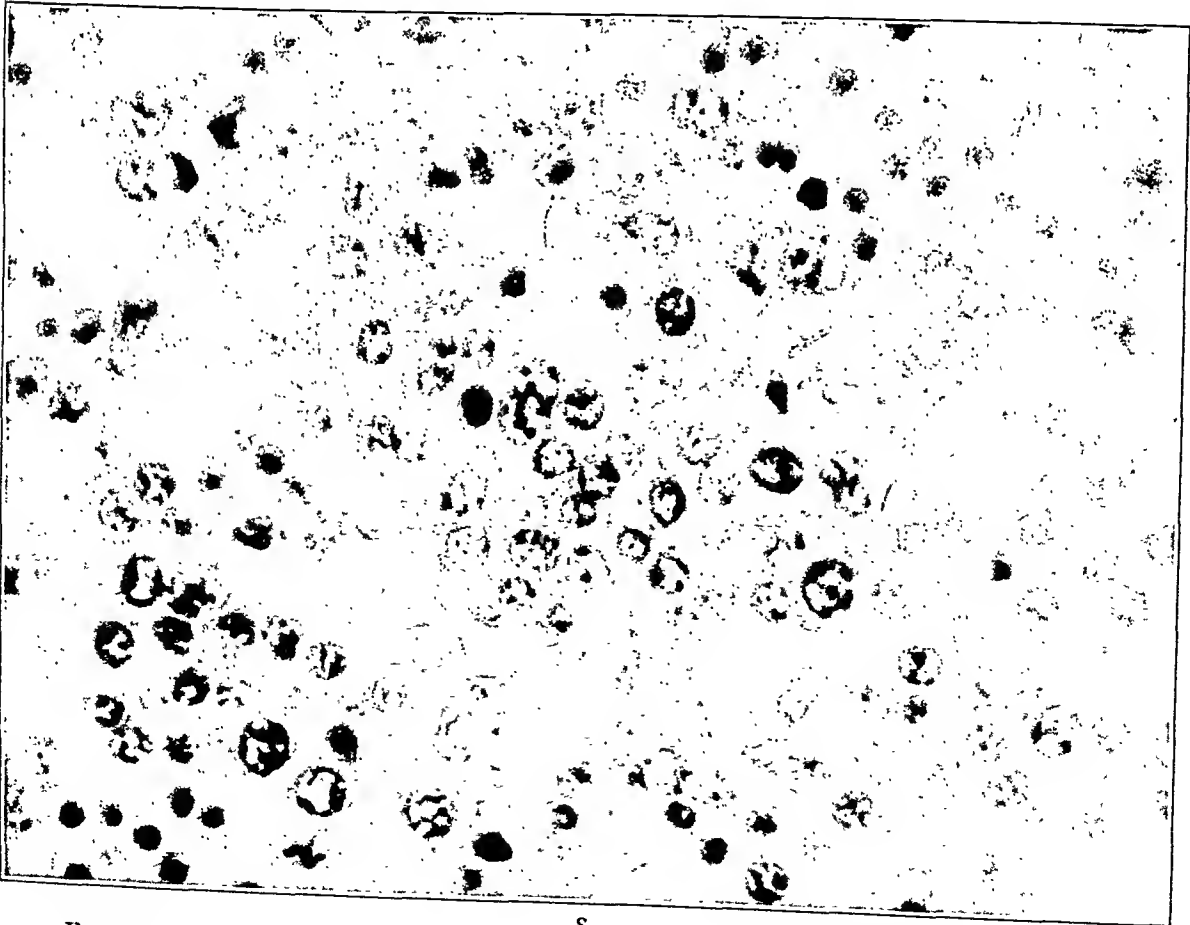
PLATE 54

FIG. 9. Case VI. Liver. The large undifferentiated cells of the hematopoietic foci stand out. The liver cords are well differentiated. The liver cells show fine masses of bile pigment. Eosin-methylene blue stain.  $\times 250$ .

FIG. 10. Case VI. The photograph was taken after completion of autopsy. The generalized edema is clearly evident.



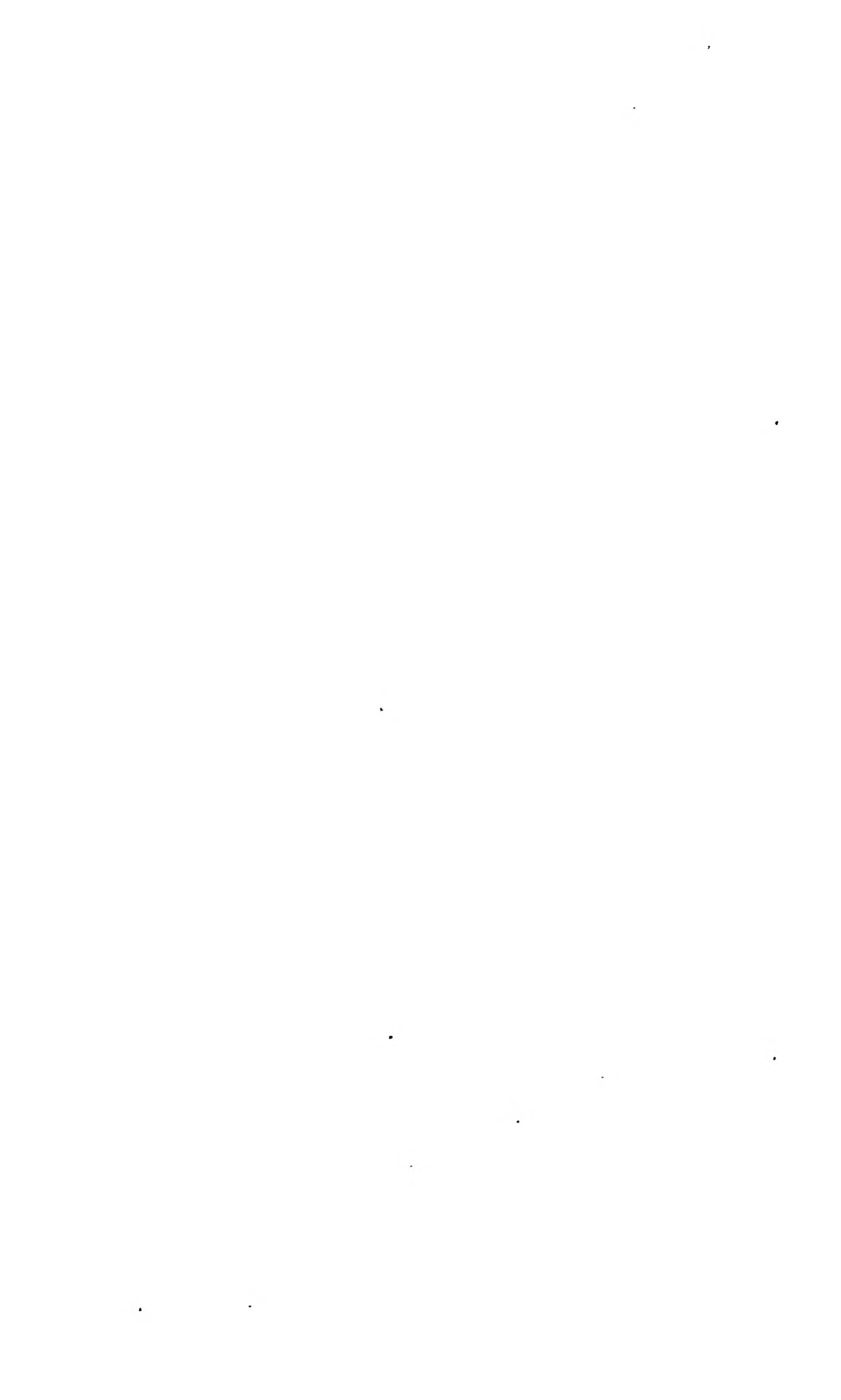
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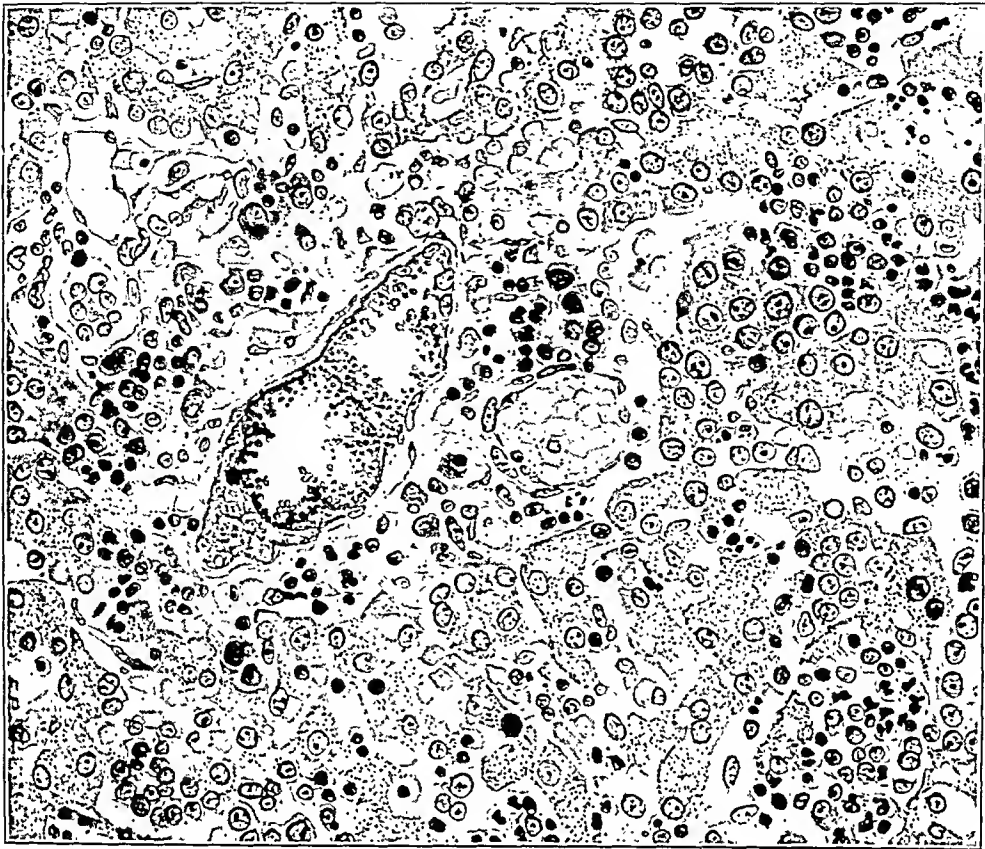


8

Ferguson

Erythroblastosis in Newly Born





9



10

Ferguson

Erythroblastosis in Newly Born



spaces are moderately edematous. A similar histological change may be found throughout the entire liver, and such a picture has been described by Rössle<sup>2</sup> as a "diffuse serous hepatitis."

In instances where the liver has been more severely injured, the liver cells in the central zones of the lobules may show dissociation necrobiosis and necrosis. The last change is not infrequently associated with an extravasation of red blood cells and an infiltration of polymorphonuclear and endothelial leucocytes.

In another group of cases, the changes within the lobule may be negligible, the outstanding lesion being almost entirely confined to the portal areas. Here one finds an acute exudative inflammatory reaction in the portal connective tissue characterized by an infiltration of polymorphonuclear leucocytes, strands of fibrin, edema and swelling of the collagen fibrils. The liver cells bordering this area may show early degenerative changes.

Occasionally one finds an almost pure lymphocytic infiltration of the portal area, a picture first described by Friedreich and von Gaffky,<sup>3</sup> and later spoken of by Virchow<sup>4</sup> as "Lymphome." Rössle regards this lesion as an inflammatory hyperplasia, in the sense of an increased resorptive activity against toxins passing from the damaged lobule into the portal areas.

Whereas we have depicted two rather distinct groups of lesions, one occurring within the lobule and the other in the portal area, we do not imply that both may not be found together — on the contrary this is a fairly common finding. However, to repeat what we have already mentioned, none of these lesions is the result of the actual presence of the streptococcus within the liver, but is due to the presence, either directly or indirectly, of a circulating toxin within the blood stream.

### ACUTE INFECTIOUS HEPATITIS

Gastou<sup>5</sup> in 1893, in his description of "foie infecté," was the first to point out that a diffuse, acute inflammation of the portal areas was not uncommonly associated with focal intralobular hepatitis, and in a child diagnosed clinically as having diphtheria, which terminated fatally, he demonstrated in the liver both an infiltration of the portal connective tissue and inflammatory foci (containing cocci in chains) within the lobules.

## STREPTOCOCCUS HEPATITIS \*

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Infectious lesions of the liver do not play as important a rôle as those of a toxic nature, but they occur in considerable variety, and some of them, syphilis and tuberculosis for instance, are of considerable importance in the pathology of this organ. It is the object of this paper to present several examples of a type of infection of the liver which may not be so infrequent as examination of the literature might lead one to believe. Furthermore, its importance lies in the fact that it may throw light on certain instances of acute yellow atrophy, and on the type of cirrhosis which may follow that lesion if recovery takes place.

The degenerative and inflammatory changes of the liver that may accompany streptococcus infection with and without a septicemia vary considerably and show no one characteristic histological lesion that may be considered as specific for this organism.

### ACUTE TOXIC HEPATITIS

The more common changes which are found in the liver in cases of streptococcus infection are of a degenerative and inflammatory nature and are usually ascribed to the effect of toxins circulating within the blood stream. These lesions may be distributed diffusely and uniformly throughout the liver, or they may appear quite irregularly. Such lesions as the latter have been described by Helly<sup>1</sup> under the name "septische Leberfleckung." This is characterized grossly by the presence of anemic-like zones throughout the liver. Histologically one sees changes in both the liver cells and endothelial cells. The former are swollen, granular, intensely stained, and frequently distended with fat. The endothelial cells are larger than normal, and show both proliferation and desquamation. The sinusoids contain less blood than the surrounding areas, and the perisinusoidal

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and phosphorus, were definitely ruled out. Smears made directly from the lesions within the liver at the time of the autopsy revealed streptococci. In view of this finding, even though organisms were not demonstrable within the fixed tissue preparations, the author felt that the lesions were probably infectious in origin.

Landé reported two cases of acute focal necrotizing hepatitis, and in both, the lesions varied in size and appeared as grayish yellow areas against a darker background. Microscopically the lesions varied in size from small foci scattered within lobules to much larger areas involving several lobules. These showed dissociation of both reticulum and liver cells, degeneration and necrosis of the liver cells, together with an infiltration of mononuclear leucocytes. Even in the most extensive areas, the vessels and bile ducts together with the interstitial tissue comprising the portal areas were moderately well preserved. Both cases were considered as infectious; streptococcus hemolyticus was recovered from the blood of one of these, but organisms were not demonstrated within the lesions of either.

Aschoff<sup>10</sup> refers to the lesions already described by Landé, and adds that similar lesions may be found in other infectious diseases as well as in streptococcus infection. Furthermore, he points out that probably many foci, often considered as pure necrosis, may be considered in this class.

Rössle, in his description of focal and acute inflammatory lesions of the liver, states that this group of lesions constitutes a relatively uncommon finding, and in autopsies in which one might anticipate these lesions, such as in cases of septicopyemias, they are usually lacking.

Thomson<sup>11</sup> speaks of a type of infectious jaundice in newborn children caused by the streptococcus being carried to the liver from the umbilicus and giving rise to an acute hepatitis. An interesting point which he brings out, and one which has a bearing on one of our cases, is that the umbilicus, although the primary point of infection, may show no external sign of inflammation.

Rolleston,<sup>12</sup> in discussing the etiology of icterus gravis, includes streptococci among the etiological agents in producing the changes within the liver, but feels that the lesion is the result of a generalized toxemia striking a liver that is already lacking in vitality, rather than the direct result of bacteria being present within the organ. He

It is with this latter lesion — a lesion described recently by Landé<sup>6</sup> as an “acute focal necrotizing hepatitis” — that we are principally interested in this report, because we believe that in some instances, but not in all, it is the result of the actual presence of the bacteria within the liver.

In the year following Gastou's report, Babes<sup>7</sup> reported four cases of fulminating streptococcus septicemia showing widespread gross and microscopic degenerative and necrotic changes in the liver. In each case streptococci were obtained from the blood, and in all cases but one these organisms were demonstrable in the liver. The histological lesion which he described in three of the cases resembled acute yellow atrophy in the very early stages, showing in addition the sinusoids distended with mononuclear cells containing streptococci. The lobules were made up of trabeculae of large, swollen, granular, eosin-staining, necrotic liver cells. In the fourth case, a patient who had been jaundiced for some time, the liver grossly resembled a later stage of acute yellow atrophy. It was small, shrivelled, soft and red. Histologically much of the parenchyma had disappeared and bile ducts had begun to proliferate and extend into the lobules. Streptococci were neither demonstrable culturally nor in the fixed preparation, although they were recovered from the heart's blood and from several other organs. Babes made no attempt to explain acute yellow atrophy on an infectious basis, but thought that perhaps occasional cases might be of infectious origin. Furthermore, in explaining the absence of streptococci in the liver in the last case he believed that the organism had produced its destructive lesion, and subsequently disappeared.

Ringel,<sup>8</sup> in reporting the pathological findings within the liver in eight fatal cases of scarlet fever, found in two of them irregularly scattered foci of necrotic liver cells infiltrated with an inflammatory exudate. Bacteria were neither isolated nor were they seen within the lesions.

Baginsky<sup>9</sup> reported an interesting case of interstitial hepatitis with widespread but isolated necrosis and inflammation of the liver parenchyma occurring in a child 10 years of age. Clinically it was a picture of a generalized septicemia of ten days' duration, and streptococci were obtained from both blood culture and liver puncture. Grossly the liver was soft, grayish yellow, cloudy and swollen. Syphilis and tuberculosis, as well as such chemical poisons as alcohol

the literature.<sup>15, 16, 17, 18, 19, 20, 21, 22</sup> In fact attempts have been made to show that any and all types of cirrhosis could be explained on an infectious basis. Today, however, such an idea seems absurd. The etiological and morphological classifications of cirrhosis have been more accurately determined, and specific lesions of the liver of a chronic progressive inflammatory nature can usually be grouped among the more common and well recognized types of cirrhosis.

Siredey<sup>23</sup> in 1886 described lesions in the liver both degenerative and inflammatory in nature in cases of diphtheria and scarlet fever, and believed that the inflammatory reaction may become chronic and account for certain instances of sclerosis found in later years. He also remarked that patients with an alcoholic history are predisposed to inflammatory lesions within the liver, stating that the alcohol may lower the vitality of the cells, making them more susceptible to infection.

A year later Mogk<sup>24</sup> reported a case of cirrhosis occurring in a young child who, eight weeks before death, suffered a very severe attack of scarlet fever. The liver at autopsy showed irregular areas of necrosis, an inflammatory exudate and a proliferation of connective tissue. The most interesting finding in this case, however, was that chains of streptococci were demonstrated within the more recent lesions.

At a pathological meeting several years later this case of Mogk's was thoroughly discussed by Schlichthorst<sup>25</sup> who, admitting the probability that the changes in the liver were part of the infectious disease, found it difficult to believe that streptococci could persist in the liver for so many weeks.

Henoch<sup>26</sup> was convinced of the important rôle infectious diseases play in the etiology of cirrhosis of the liver. He observed in the very severe cases of measles and scarlet fever signs and symptoms indicative of pathology within the liver. These lesions, he pointed out, could either completely disappear with full restoration of the liver, or could persist long after the acute infection, as an interstitial hepatitis. Histologically he found in such cases a moderate hepatosis, proliferation of the portal connective tissue and dilatation of the small ducts, and looked upon this form of hepatitis as being quite capable of developing into a true cirrhosis.

Folger<sup>27</sup> reported an unusual case of hypertrophic cirrhosis of the liver in a child who had been jaundiced for weeks. The liver was

states that various organisms have been found within the liver but none so constantly as to justify definite conclusions.

One obtains little aid in the study of these acute, non-suppurative, diffuse and focal forms of hepatitis involving the parenchyma of the liver from textbooks of pathology and medicine. Certainly one gains the impression that if streptococci reach the liver they either produce no demonstrable lesion, or an abscess results. Karsner,<sup>13</sup> however, does devote a paragraph to acute non-suppurative inflammation of the liver, in which he describes two types. In one the liver shows cloudly swelling, and histologically one finds an acute polymorphonuclear leucocytic exudate in the portal areas: in the second type, described as acute interstitial hepatitis in contrast to the first which he calls acute parenchymatous, one finds mononuclear leucocytes and lymphocytes instead of polymorphonuclear leucocytes.

The production of abscesses within the liver by the streptococcus is mentioned in almost all textbooks of pathology, and although not a common finding at autopsy it is one that is generally accepted as being beyond dispute. One finds in the literature isolated reports of abscess formation following streptococcus infection; perhaps among the earliest recorded cases is one by Roger<sup>14</sup> in 1896. The patient, a young woman 30 years of age, complained of severe abdominal pain demanding surgical interference. A large abscess was revealed in the tubo-ovarian region from which streptococci were grown in pure culture. The convalescence was poor and the patient died within a few days. An autopsy revealed abscesses in the liver in addition to the pelvic condition. Cultures taken from both sources demonstrated an organism in pure culture similar to that which was obtained a few days earlier at the operating table.

In the more recent literature mention is made by Thomson<sup>11</sup> of the same conditions occurring in children following infection of the umbilical vein.

Before describing the cases which we have found of this acute focal and diffuse non-suppurative type of hepatitis, we wish to consider an entirely different form of hepatitis that is essentially chronic and progressive.

#### CHRONIC INFECTIOUS HEPATITIS

Suggestions of a chronic progressive inflammatory process in the liver going on to form a true cirrhosis and ascribed to streptococci colon bacilli and other bacteria are not infrequently encountered in

An attempt to ascribe certain chronic inflammatory lesions of the liver to streptococci, acting locally, is of course open to criticism even where the organism may be demonstrable within the lesions of the liver, since such a bacteriological finding could occur in a terminal bacteremia. Cases assumed to be the result of streptococcus infection, purely on the basis of a clinical history — such cases in which the organisms are not demonstrable, the supposition being that they have died and disappeared — are subject to even greater criticism and can be accepted only with reserve.

### HEALED INFECTIOUS HEPATITIS

The last point which we must now consider is the gross and histological changes which one may find in the healed stages of these acute and chronic inflammatory processes within the liver. Where the acute lesions are small there is evidence to show that there is probably complete structural and functional restoration; yet as Rössle says, "we know too little about the fate of these small areas of necrosis and the associated liver changes that may appear with various infectious diseases." The large areas in which all of the liver cells in one or more lobules have been destroyed probably show incomplete regeneration, and heal by sclerosis. Such healed lesions are characterized by the presence of one or more rather sharply circumscribed and irregularly distributed areas composed of connective tissue, bile ducts and regenerated liver tissue; the latter in some cases is entirely absent. This picture, though usually focal in distribution, resembles very closely the healed stage of true acute yellow atrophy of non-infectious origin.

Apropos of this, is an unusual case of cirrhosis, reported several years ago by Rössle,<sup>29</sup> occurring in an elderly male. The liver was quite normal, except for a single isolated area of sclerosis about the size of the palm of the hand lying on the anterior surface of the right lobe. This was irregular, yellowish brown and extended into the liver several finger breadths. Sections from different parts of the liver were carefully examined histologically and except in the areas of sclerosis showed no noteworthy change. The lesion itself showed definite cirrhosis, with reconstruction of the remaining liver parenchyma, increase in connective tissue and bile ducts and a small round cell infiltration. No visible explanation was found for this rather

definitely cirrhotic, and showed advanced sclerosis and a massive production of small bile ducts. Streptococci were demonstrable in the liver and other organs, but were accepted by Folger with considerable doubt as having any direct bearing on the lesion within the liver.

Bingel <sup>8</sup> firmly believed that certain cases of cirrhosis in children, alcohol and syphilis being excluded, could be directly linked up with changes in the liver which may occur in severe epidemics of scarlet fever. He reported a case in a young child, 9 years of age, who had recently suffered a very severe angina, probably of scarlet fever origin, accompanied by pain in the right upper quadrant. The convalescence was poor and seven months later jaundice appeared, together with gastric distress and fever. A few days later the child died. The liver was uniformly firm, sclerotic and irregularly lobulated. Microscopically it resembled somewhat the alcoholic type of cirrhosis, but in addition the fibrosis extended quite often between groups of liver cells. There was a reconstruction of the liver parenchyma, marked increase in connective tissue and a somewhat irregularly distributed, small, round cell infiltration.

Very recently Moon <sup>28</sup> reported two cases showing a progressive type of cirrhosis which he considered as being infectious in origin. One of these occurred in a patient, aged 12 years, who had been diagnosed clinically as having "Banti's disease." The spleen and liver were of about equal size, each weighing just under 1000 gm. The liver, which was firm and nodular, was diagnosed as atrophic cirrhosis. Histologically a section stained for bacteria showed cocci in pairs throughout this organ. The autopsy unfortunately was done a considerable number of hours postmortem; thereby lessening to some extent the importance attributed to these organisms within the liver. The second case was in a boy 14 years of age. The family history is worthy of note in that several of the children had already died from cirrhosis of the liver. This child's spleen and liver were large, and in addition he showed marked anemia, moderate leucopenia, increasing ascites and shortly before death a slight degree of jaundice. No clinical diagnosis was made. At autopsy the liver was large, firm, and showed a hobnail granular surface. Sections of liver tissue showed cocci in areas of more recent degeneration and necrosis, and in addition a pure culture of streptococcus hemolyticus was obtained from the liver at the time of the autopsy.



streptococci. The wall of the umbilical vein was thickened and the lumen contained pus.

The liver, weight 179 gm., was enlarged and smooth. The sinus venosus was patent, though the wall was thickened and surrounded by a wide area of necrotic tissue extending from the wall into the liver substance. Smaller areas, yellowish red and varying in size from a pinhead to 1 cm. in diameter, were scattered throughout the liver.

The spleen, weight 38 gm., was large, soft and of the septic type.

No noteworthy lesions were found in the heart, lungs and other viscera. The bacteriological examination from both peritoneal cavity and liver was positive for streptococcus hemolyticus.

*Anatomical Diagnoses:* Acute infectious hepatitis; acute peritonitis; pyophlebitis of the umbilical vein; cellulitis of the right wrist and omphalitis.

### HISTOLOGICAL EXAMINATION

The umbilical vein shows an inflammatory reaction with a fibrinous thrombus attached to its inner surface, and an infiltration of endothelial leucocytes, lymphocytes and plasma cells in its wall.

Within the liver some of the branches of the portal vein are distended with endothelial leucocytes containing numerous streptococci, together with fibrin, polymorphonuclear leucocytes and free streptococci. Many of the smallest branches of the portal vein contain endothelial leucocytes with many streptococci within them.

The liver lobules show scattered foci of hematopoiesis. The outstanding lesion present consists of necrotic liver cells occurring singly and in small groups. They extend to the portal vessels and to the central veins but are most numerous in the intermediate zones. Some lobules show many more of these lesions than others. The necrotic cells tend to stain deeply with eosin and the nuclei are more or less pyknotic. Others have lost their nuclei and are being surrounded or invaded by endothelial leucocytes. In certain areas which may involve one or more complete lobules, all of the liver cells have disappeared, leaving only the stroma infiltrated with numerous endothelial leucocytes. There is no evidence in any of the lobules of a toxic central necrosis, and no abscesses are present.

The most noticeable feature in the sections stained by the Gram-Weigert method is the presence of large numbers of streptococci, chiefly in the endothelial cells lining the sinusoids, but also to some extent within the vessels. In the areas where all the liver cells have been killed off and have disappeared, the stroma is infiltrated with endothelial leucocytes containing fairly numerous streptococci.

rare occurrence and the author suggested that it was probably a type of cirrhosis that could be explained on the basis of an embolic toxic-infectious process.

### MATERIAL FOR STUDY

The material which forms the basis for this work was obtained from several of the larger hospitals of Boston, and the cases which are reported below have been selected from several thousand autopsies. We have included only those cases of streptococcus hepatitis which can best be explained as the result of the actual presence of the organism within the liver, and the five cases selected are fairly representative of the types of pathology one may encounter. We have purposely omitted those showing the more common and well recognized degenerative and inflammatory changes so often seen in instances of generalized toxemia of streptococcus origin.

We have considered the lesions in the liver as occurring in three rather characteristic forms: the acute stage with necrosis of liver cells accompanied by a cellular exudate; a chronic lesion showing degeneration and necrosis on the one hand and active proliferation of liver cells, bile ducts and connective tissue on the other; and lastly the healed stage, from which all signs of an active inflammatory reaction have disappeared. As examples of the acute lesion three cases are fully reported. The remaining two cases represent the chronic and healed lesions. We are quite aware of the criticism that may be directed against these latter cases, and for this reason they are presented not as definitely proved examples of what the streptococcus can do, but only as possibilities that may result when the process becomes chronic, and lastly when it has entirely healed.

### CASE REPORTS

CASE 1. (C. H. A. 28-30), an apparently healthy, white male infant, aged 8 days, developed an abscess near the right wrist. Two days later this was incised and drained. On the third day after the operation the child suddenly developed difficulty in breathing, cried almost continually, and passed five loose green stools during that night. The following morning he was admitted to the hospital dangerously ill. In addition to the lesion on the wrist, an examination revealed signs of bronchopneumonia, a distended abdomen and a protruding umbilicus covered with a pigmented crust. Death occurred a few hours after admission, and an autopsy was performed one and a half hours postmortem.

The peritoneal cavity contained an excess of amber-colored fluid in which were clumps of thick, white, purulent material. Smears of this revealed chains of

ducts in the portal systems are prominent, but they have not yet begun to grow toward the centers of the lobules. Around them are a few polymorphonuclear leucocytes, eosinophiles and lymphocytes.

Examination for organisms in the fixed preparations, especially for streptococci, was entirely negative.

The decidedly focal character of the two lesions present in the liver strongly suggests that they are of infectious rather than toxic origin. The lesion is in the reparative stage and the causal agent has been destroyed and removed. There remain two foci showing the early healing stages of acute yellow atrophy. In time these would have terminated in areas of sclerosis.

CASE 3. (B. C. H. 01-46), a white female, aged 20 years, was operated on Feb. 2, 1901 and the left ovary removed. On March 16, about six weeks later, at a second operation the right ovary and tube were excised and a diagnosis of acute purulent salpingitis made. Death occurred a week after this second operation and at the postmortem examination made twelve hours later, acute salpingitis of the left tube, a pelvic abscess, and a localized peritonitis of the pelvis were found.

The liver, weight 2320 gm., was much enlarged, smooth and mottled yellowish brown. On section the middle portions of the lobules were yellow and surrounded by narrow red zones.

Cultures from the heart's blood, spleen, kidneys and liver showed a streptococcus.

*Anatomical Diagnoses:* Pelvic peritonitis, septicemia.

### HISTOLOGICAL EXAMINATION

Many of the liver cells contain small to medium sized fat vacuoles; occasionally single large vacuoles are present. Necrosis of liver cells is extensive and diffuse and involves an irregular zone about each portal area from two to ten cells in width, but as a rule leaves one or two rows of cells adjacent to the portal area comparatively uninjured. Viewed in relation to the central vein of the lobule it could be called a zonal necrosis involving principally the periphery of the lobule. Occasionally the necrosis reaches the portal connective tissue, less often it extends here and there to the central vein. There is no evidence anywhere of a toxic central necrosis, even in its earliest stages. The cytoplasm of the necrotic cells is finely granular and strongly eosinophilic. In an occasional normal liver cell adjoining the necrotic zone a mitotic figure can be found. In one section three were grouped closely together.

In this case the necrosis of the liver cells and the inflammatory reaction are evidently due to the direct action of the toxin liberated by the organisms present in the lesion. This toxin has destroyed the more highly specialized liver cells, leaving the endothelial cells and fibroblasts relatively uninjured.

If the child had overcome this infection and lived, the streptococci and necrotic liver cells would have been removed and the histological picture would then suggest a rather late stage of acute yellow atrophy. In many places only the stroma and portal vessels would have remained, whereas in areas where liver cells had escaped necrosis, regeneration would have occurred. The end result would have been a cirrhosis corresponding to that so often seen following acute toxic hepatitis.

CASE 2. (U 25-23), a female infant who had suffered a prolonged and difficult delivery and died on the fifth day after birth. A postmortem examination disclosed a hemorrhage into the cerebellar fossa, with extension down the spinal canal and out into the loose tissue of the neck.

The liver, weight 150 gm., was normal in size, shape and consistence and dark reddish brown. On the upper surface of the right lobe were two round areas, one 3 cm. in diameter and the other 2 cm. Both were slightly depressed beneath the normal surface, and yellowish brown.

On section these depressed areas were seen to extend about 1.5 cm. into the liver parenchyma. Their yellowish brown cut surfaces were striated with dark red lines suggesting distended capillaries. In consistence, these areas were distinctly softer than the surrounding tissue.

A culture from the heart's blood yielded streptococcus hemolyticus in pure culture.

*Anatomical Diagnoses:* Infratentorial hemorrhage into cerebellar fossa. Focal necrosis of liver.

### HISTOLOGICAL EXAMINATION

Sections from the greater portion of the liver show a few small foci of hematopoiesis but nothing abnormal beyond the presence of small and medium sized fat droplets in some of the liver cells. There is no toxic central necrosis. The two lesions described in the gross examination show a very different condition. Necrosis of liver cells is extensive and in many of the lobules all of them have been killed. In other lobules they remain around central or portal vessels, or are scattered in small groups within the lobule. Masses of necrotic cells are still present and are slowly being invaded and surrounded by endothelial leucocytes and gradually dissolved. The terminal bile

At autopsy the tissues were all deeply jaundiced, the abdomen was distended and the peritoneal cavity contained 6000 cc. of slightly cloudy yellowish fluid containing flakes of fibrin.

The liver, weight 1920 gm., extended 1 cm. below the costal margin. Old adhesions joined the anterior surface of the liver to the under surface of the diaphragm. The left lobe was reduced to a small scarred puckered mass of connective tissue lying to the left of the coronary ligament, which, when sectioned, revealed scar tissue, blood vessels and large bile ducts.

The right lobe was yellowish in color with a fairly nodular surface. It cut with increased resistance, exposing on the fresh surfaces nodules of deep golden yellow which changed to green on exposure to air.

The remaining viscera, with the exception of the spleen which was enlarged and rather lax and weighed 400 gm., showed no noteworthy changes.

*Anatomical Diagnoses:* Cirrhosis of the liver, peritonitis, ascites and jaundice.

### HISTOLOGICAL EXAMINATION

The liver presents a most unusual appearance. The original lobular architecture is almost entirely replaced by wide interlacing tracts of proliferating bile ducts and connective tissue which isolate small nodules of liver cells composed partly of remnants of former lobules together with regenerated trabeculae showing no orderly structure. These young bile ducts form a most intricate meshwork of channels among themselves, often encircling small groups of liver cells. The bile ducts are definitely abnormal, resembling somewhat the structures seen in primary bile duct tumors. The cells and nuclei are parallel with the lumina, both are distinctly elongated, instead of being rather cuboidal with the nuclei at right angles to the lumina. Occasional clusters of streptococci can be found among the liver cells, some are extracellular, others intracellular, apparently within the cytoplasm of endothelial cells lining the sinusoids. In other fields are small groups of degenerating and necrotic liver cells which are infiltrated with polymorphonuclear and endothelial leucocytes. The interstitial tissue is increased, especially about the new formed bile ducts and also, although to a lesser degree, among the regenerated liver cells. The stroma everywhere is infiltrated with neutrophilic leucocytes, endothelial leucocytes, lymphocytes, occasional eosinophiles and nests of plasma cells.

The striking feature of this case is the extraordinary number of bile ducts present. They are so prominent that they suggest the possibility of a tumor but evidently are not. Small patches of a somewhat similar bile duct formation occur in other forms of cir-

The necrotic cells are surrounded and to some extent invaded by polymorphonuclear and endothelial leucocytes. The former often collect in considerable numbers but no abscesses are found. They are present also in the portal connective tissue together with endothelial cells and lymphocytes. Here and there branches of the portal vein are distended with clots, evidently of postmortem origin, consisting of fibrin, polymorphonuclear and endothelial leucocytes. Inspissated bile is present in some of the bile capillaries near the central vein.

A striking feature of this lesion is the presence of masses of streptococci most often within and adjoining the zones of necrotic liver cells. They are found in endothelial cells lining the sinusoids and also in the vessels, extending along them and often filling them.

In this liver we have a lesion uniformly distributed in every lobule, in close relation to the portal areas but occasionally reaching the central vein. This uniformity of distribution would suggest a toxic origin. On the other hand, the numerous clumps of cocci situated in the endothelial cells and in the sinusoids adjoining the affected liver cells strongly favor the view that, in part at least, they bear a causal relationship to the necrotic cells.

The clinical history of this case resembles very closely the case reported by Roger <sup>14</sup> in 1896. In his case, however, the pathological anatomy differed in that the liver was riddled with abscesses.

There is another explanation for this zonal necrosis based on experimental work done by Opie <sup>30</sup> which will be discussed more fully below. He found that by producing a bacteremia in an animal whose liver he had previously injured, he invariably produced a rather characteristic midzonal form of necrosis. Certainly the more common severe lesion caused by the streptococcus toxin is a central necrosis, and of that there is not the slightest evidence in this liver.

CASE 4. (P. M. H. 970, M 1153), a white male, 56 years of age, was operated upon for appendicitis and made an uneventful recovery. Ten months later the gall-bladder containing two concretions was removed. The liver was reported to be small and a piece was excised which histologically showed nothing abnormal. Seven weeks later the patient developed chills and fever which persisted for a month. Jaundice later appeared; and finally ascites developed which required tapping on two occasions. At this time the liver was observed to be definitely enlarged. The patient's condition progressed gradually and eight and a half months after the operation on the gall-bladder he died with an extensive cirrhosis of the liver.

## REPORT OF EXPERIMENTAL WORK

If we are willing to postulate that streptococci acting locally can produce a definite inflammatory reaction in the liver with degeneration and necrosis of the parenchyma, then one might ask what experimental evidence we have to substantiate such a claim. Furthermore, can we assume in certain cases of streptococcus infection in which one finds inflammatory foci within the liver, but no organisms, that such lesions are actually infectious in origin, only the organisms have been rapidly killed and removed?

We have employed rabbits in our experimental work, but there are certain dangers in attempting to correlate pathological lesions produced in lower animals with those seen in man, in that we have here two biological systems of quite different constitution. The problem becomes still more complicated when we add to these a third living organism — namely an organism so complex and variable as the streptococcus. In attempting, therefore, to answer these two questions, namely, can streptococci produce similar changes in animals, and how long may the organisms survive within these lesions, we report the results of our work with considerable reserve.

Among the earliest investigators in this field of research was Wyssokowitsch<sup>31</sup> who as early as 1886 showed that the endothelial cells of the liver were capable of phagocytosis and would take up bacteria that were injected into the circulating blood.

Many years later this work of Wyssokowitsch's was repeated by Nathan,<sup>32</sup> using not alone bacteria, but collargol and other substances as well. He verified the earlier work on phagocytosis by the Kupffer cells, and demonstrated an active proliferation of these cells followed by a desquamation into the circulating blood.

Roger<sup>14</sup> in 1896, after isolating a streptococcus from abscesses within the liver, made an emulsion with saline and injected this subcutaneously and intravenously into rabbits, but produced no marked demonstrable reaction.

Weaver<sup>33</sup> in 1900 produced a type of cirrhosis in guinea pigs by inoculating a strain of *B. coli* into the portal vein. This type of cirrhosis was characterized by an increase in bile ducts and perilobular connective tissue. His results indicate how critical one must be in interpreting the results obtained in lower animals, because the same organism produced absolutely no lesions in rabbits.

hosis such as the pigment and syphilitic types, but to nothing like the extent and amount present in this case.

In the relatively common type of toxic cirrhosis following acute yellow atrophy, the bile ducts at the periphery of each lobule grow for a certain distance toward the center and then stop. In the adult they do not produce liver cells and they do not extend indefinitely.

The history of this case strongly suggests infection of the liver. "Seven weeks after cholecystectomy the patient had chills and fever which persisted for a month. After two months he became jaundiced, and one month later developed ascites. He died from cirrhosis of the liver eight and a half months after the operation of cholecystectomy."

Unfortunately the liver was not cultured at the time of the autopsy. The chains of cocci seen in the stained sections may represent simply a terminal bacteremia, or, and what seems to us not improbable, they may have been present in the liver for weeks, possibly having gained entrance to the organ at the time of the cholecystectomy. There seems to be no other way to explain it. It would mean a chronic lesion due to a streptococcus of moderate virulence causing widespread, but not extensive and rapid necrosis.

CASE 5. (B. C. H. 98-211), a young woman who died of pernicious anemia. The immediate clinical history is irrelevant insofar as it has no bearing on an old healed inflammatory process which was found within the liver.

The liver was of normal size and color, but revealed both beneath the capsule and on the cut surface minute grayish areas suggesting small scars.

### HISTOLOGICAL EXAMINATION

These small scar-like areas consisted of contracted lobules containing numerous bile ducts but no liver cells. They strongly suggest healed patches of acute yellow atrophy and so far as can be determined from their size, shape, isolation and distribution, are much more likely to have been of infectious than of toxic origin. The lesions at first suggested multiple adenomas of bile duct derivation, but careful study of them later disclosed the contracted lobular arrangement with the portal vessels still evident.



showed that at the end of one hundred and twelve hours the cocci were completely destroyed.

We began our experimental work using a group of guinea pigs, but because the livers of these animals were practically refractory to infection, it was necessary to select rabbits, which proved to be more satisfactory.

A pure culture of streptococcus hemolyticus which had been isolated from the throat of a patient with scarlet fever was used throughout the experiments. On blood agar the colonies were quite typical, being small, gray and opaque and surrounded by a wide clear ring of hemolysis.

A saline suspension was made from a growth on blood agar slants as we wished to inject the organism as free from toxin as possible. The quantity injected varied from 3 to 4 cc. of a moderately heavy uniform suspension.

As a control, an equal quantity of a similar suspension that had been heated to 60° C for one hour, and proved sterile, was introduced into the rabbit's liver under precisely the same conditions. These animals lived and showed neither gross nor histological lesions within the liver.

The operating technique was simplified as much as possible. The abdomen was shaved and using aseptic precautions a small opening was made into the peritoneal cavity. A loop of the small intestine was drawn through the opening, thereby exposing branches of the mesenteric artery and portal vein. The vein was freed for about 1 cm. from the surrounding fat and connective tissue and ligated at the distal end of this exposed section. A second ligature was loosely tied about the vein proximal to the first. The next step was to inject the organisms into the vein, and for this a small Luer syringe with a No. 24 gauge needle proved most suitable. Just before withdrawing the needle the proximal ligature was tightly tied. The intestines were replaced and the abdominal wall closed with a double row of continuous sutures.

The first rabbit died after eighteen hours, the second after twenty. The third, which made a good recovery and appeared quite healthy, was killed at the end of forty-eight hours. The fourth rabbit also made a good recovery but was killed after five days.

Blood cultures taken from the ear veins of these animals at the end of twelve hours showed streptococci in pure culture. Further cultures taken after forty-eight and seventy-two hours were negative.

A year later Hektoen<sup>34</sup> reported certain results, confirming the work of Weaver. He produced a similar lesion, using a second organism belonging to the *B. diphtheria* group, and was able to demonstrate the organisms within the early lesions.

As far as we can determine from a review of the literature, Opie<sup>30</sup> was the first to attempt to produce lesions experimentally within the liver by injecting streptococci. When he injected a suspension of streptococci intravenously into dogs, the only appreciable change he found in the liver was a slight deposition of fat within the liver cells. However, if he first inoculated dogs with a small amount of chloroform or phosphorus — an amount which he previously determined to be incapable of producing destructive lesions within the liver — and then followed this a few days later with an intravenous injection of streptococci, he invariably produced extensive midzonal and central necrosis — a type of lesion resembling that of acute yellow atrophy. He explained these lesions on the basis of a combined intoxication, making no attempt to link up the changes with the presence of organisms within the liver.

Recently Moon<sup>28</sup> in an attempt to substantiate his claim that a strain of streptococcus hemolyticus, which he had isolated from the liver of a young child with cirrhosis was the causative agent in this disease, injected a suspension subcutaneously and intraperitoneally into rabbits and produced degenerative lesions in the liver in which he demonstrated the organisms.

Regarding the second question relative to the period of survival of organisms within inflammatory lesions in the liver, some information is to be found in a report by Schwarz.<sup>35</sup> This worker injected into mice an organism of the diphtheroid group which he had isolated from a child's liver. He killed these mice at intervals of one to seven days following the injection. After twenty-four hours the liver was riddled with minute inflammatory foci teeming with organisms; as the interval of time increased, the number of organisms diminished, and after seven days he was unable to demonstrate organisms within the lesion.

Kyes<sup>36</sup> studied the fate of pneumococci after intravenous inoculation into the pigeon, which is naturally resistant to this organism. He made careful studies of various organs and found that the organisms were quickly removed from the circulating blood and localized within the endothelial cells of both spleen and liver. In addition he

ings, other areas were traversed by thin yellow lines, and still others were mottled with small yellowish foci which varied in size and contour. After five days, the liver was normal in size, moderately firm and dark reddish brown. The surface was smooth, except for three yellowish depressed areas, each about 2 to 3 mm. in diameter.

*Microscopic Examination:* In Rabbit 1, dead at the end of eighteen hours, one finds chains of cocci up to a dozen or more in many of the endothelial cells lining the sinusoids. The reaction to these consists in an accumulation of polymorphonuclear and endothelial leucocytes, together with small clumps of fibrin within the vessels. Where leucocytes have clustered in adjoining sinusoids, the isolated liver cells often show necrobiotic changes and necrosis. Such lesions are found in any part of the lobule, even adjoining the hepatic vein, but they occur most abundantly at the periphery of the lobules close to the portal vessels. These peripheral lesions may suggest in their extent and distribution, small zones of infarction, but the inflammatory reaction which is uniformly distributed throughout this damaged area differentiates them clearly from bland areas of infarction.

In Rabbit 2, dead at the end of twenty hours, streptococci are more difficult to find. The lesions are more numerous and a little larger. Many of the necrotic liver cells have already disappeared, and their places are occupied by minute islet-like collections of endothelial leucocytes.

In Rabbit 3, killed forty-eight hours after the injection, numerous large and small lesions are present. Some nearly equal the size of a lobule. These large lesions are composed of necrotic liver cells among which polymorphonuclear and endothelial leucocytes are invading and digesting the cellular débris. At the periphery of these lesions the necrotic liver cells have largely disappeared, and here accumulations of endothelial leucocytes are more prominent than centrally where the polymorphonuclear leucocytes are in greater evidence. The smaller lesions represent a later stage in this reparative process, and are merely nests of endothelial leucocytes which have removed the dead liver cells. A very few streptococci can still be found in a few of the lesions.

In Rabbit 4, killed after five days, only small lesions are present. They consist of accumulations of endothelial leucocytes and signify a late stage of repair. Either the lesions were originally very small,

The autopsy blood cultures from the first and second rabbits, which had died at the end of eighteen and twenty hours respectively, were sterile. Cultures taken directly from the liver in both cases yielded a few typical colonies on a blood agar plate. Smears made from scrapings of the freshly cut surfaces of both livers, showed in addition to liver cells, chains of cocci and many polymorphonuclear leucocytes. A blood culture taken from the third rabbit at the time of the autopsy forty-eight hours after the injection was sterile, while only three colonies were grown on a blood agar plate after being heavily streaked with a swab that had been inserted deeply into the liver parenchyma. A smear made directly from the liver at this time showed liver cells, cellular débris, polymorphonuclear and endothelial leucocytes, and a few poorly stained and questionable clusters of organisms suggesting streptococci.

The fourth rabbit, from which sterile blood cultures had been obtained at forty-eight and seventy-two hours after the injection, was autopsied at the end of five days. Cultures taken from the heart, and liver, as well as smears from the latter organ revealed no streptococci.

In summing up our bacteriological results we find that at twenty-four hours living organisms had to a great extent disappeared from the circulating blood and the liver; at forty-eight hours the number of viable streptococci in the liver was practically negligible, and lastly at the end of five days all cultures from different sources, as well as smears taken directly from the liver showed no trace of streptococci.

The rapidity with which streptococci have been destroyed in the liver may largely explain some of the histological characteristics of the lesions, and particularly the fact which has been previously stressed in this paper that it has often been quite impossible to demonstrate organisms histologically in the lesions.

*Gross Examination:* Within the first twenty-four hours there was little to suggest any severe injury to the liver. The organ was normal in size, the capsule smooth and the cut surface uniformly congested and rather soft. In the forty-eight hour animal, small areas beneath the capsule showed up as a yellowish stippling or a very fine network of delicate lines that were slightly raised above the surrounding liver tissue. The cut surface varied considerably in different lobes; some areas showed simply congestion, edema, and a loss of the finer mark-

show corresponding clinical signs and symptoms, with jaundice and increased bilirubin in the circulating blood.

Schelenz<sup>38</sup> several years later followed up this work of Hildebrandt, and reported that the liver would appear to be more severely affected in some epidemics of scarlet fever than in others. This simulates an observation which has been made repeatedly in regard to inflammatory lesions of the kidney complicating scarlet fever. This investigator reported a fatal case of scarlet fever in a young child who showed a very high urobilin excretion. The liver parenchyma revealed a slight degenerative change, and a diffuse interstitial hepatitis.

In cases of streptococcus infection there are usually no constant clinical signs and symptoms referable to functional or morphological changes within the liver. That is, the liver is involved to such a slight degree as to be clinically unrecognizable.

Where the liver is damaged it is probable that the secretion of urobilin is not the only functional alteration of secretion that occurs in the more severe infections. Smyth and Whipple<sup>39</sup> have demonstrated the marked influence that mild chloroform poisoning has on bile salt secretion. Dogs, which had received small doses of chloroform, did not show the slightest clinical indisposition, and yet the bile salt secretion was greatly reduced. Microscopic examination of the livers at this time showed only very slight degenerative changes within the liver cells in the central zones of the lobules.

Whipple and Smith<sup>40</sup> have indicated that an important function of the liver cell is to group together the amino acids to join the precursors of hemoglobin which in turn are utilized by the marrow cells in turning out the finished red blood cells into the circulation. This function of the liver cell, like that of bile salt secretion and the secretion of urobilin and bilirubin, is diminished in liver cells showing slight degenerative changes and would therefore probably be altered in streptococcus hepatitis.

## DISCUSSION

One of the most interesting characteristics of the liver is the resistance it shows to infection. Indeed how often at autopsy one sees acute suppurative inflammatory lesions in different parts of the body, severe septicemias and septicopyemias, diffuse inflammatory

or neighboring liver cells have regenerated and replaced those that were destroyed.

In summarizing these histological lesions we find that they are essentially destructive and focal in character, and in none of the livers was there a suggestion of a lesion uniformly limited to the central zones of all lobules such as is seen in toxic hepatitis resulting from chemical intoxication or severe bacterial toxemias. In other words we are dealing with a pathological condition of the liver in which the injury is the result of the immediate presence of the organisms within the lesions themselves.

### PATHOLOGICAL PHYSIOLOGY

Before entering into a general discussion of inflammatory lesions within the liver, it seems not at all irrelevant at this point to say a word about the pathological physiology of the liver in cases of streptococcus infection. Since this organ constitutes the largest gland of internal metabolism within the body and is directly or indirectly involved in the metabolism of proteins, fats and carbohydrates, any alteration in function of the liver cell, with or without histological signs, might be considered as sufficient to interfere with the metabolism of any one or all of these substances. We shall not go into this phase of the problem fully, but merely mention a few of the important functional changes which at times are manifested as clinical signs and symptoms.

Hildebrandt <sup>37</sup> in 1910, made the observation that many cases of scarlet fever showed an appreciable increase in the urobilin content of the urine. Such patients may show no trace of jaundice and no demonstrable bilirubinuria. He considered two possibilities in explaining this urobilinuria: first and more important as the result of a functional insufficiency on the part of the liver cell, and secondly, though probably merely as a contributory factor, by the increased destruction of red blood cells. He reported the findings in one liver from one case which terminated fatally. This organ was large, swollen and edematous, and microscopically revealed scattered patches of necrosis. He described these changes as a form of "parenchymatous hepatitis," and quoted Litter who believed that in instances where destruction was more widespread the liver would simulate the picture of acute yellow atrophy and the patient would

light is thrown on the interpretation of the rarity with which one can demonstrate organisms within the lesions in man.

It should be pointed out clearly that absolutely no attempt has been made to explain cirrhosis of the liver in its broad sense, as a chronic or healed inflammatory lesion of infectious origin. On the contrary, the points that are particularly emphasized are first that an acute inflammation can occur in the liver as a result of the actual presence of streptococci within this organ; and second, that with extensive destruction of liver cells, followed by a reparative proliferation of connective tissue and bile ducts, wide tracts of sclerosis can be produced, giving a picture which resembles in many respects the healed stages of acute yellow atrophy.

### SUMMARY

1. The more common inflammatory changes in the liver in cases of streptococcus infection with and without a septicemia are described.

2. Emphasis is laid on a less common lesion of which three cases are given in detail. This is characterized by focal or diffuse areas of liver tissue showing necrobiotic changes and necrosis, infiltrated with an inflammatory exudate. A Gram-Weigert stain shows streptococci in large numbers in the lesions of two of these livers.

3. The similarity of this lesion to the histological picture at times encountered in acute yellow atrophy is discussed, and the suggestion is made that a careful bacteriological search of the liver in the fixed preparation together with a culture of the liver at the time of the autopsy might reveal bacteria within the lesions more commonly than is suspected — particularly in those cases of so-called acute yellow atrophy showing a very irregular distribution of the lesion — a condition that is extremely difficult to explain purely on the basis of a circulating toxin in the blood.

4. Another case is described with a chronic inflammatory reaction within the liver, showing on the one hand degeneration, necrosis, exudation and bacteria, and on the other a very active proliferation of bile ducts and connective tissue. This case is presented more for discussion than as a proved case of chronic progressive cirrhosis of infectious origin.

reactions within the gastro-intestinal tract, sloughing tumors and ragged ulcers — and yet the liver apparently unharmed. Certainly we may say that the manner in which the liver handles massive infection is scarcely to be seen in any other organ in the body. This very fact, namely the rarity of inflammatory lesions within the liver, coupled with the rather unusual character of these lesions when they do occur, makes the study of this organ one of the most interesting problems in general pathology.

What structures are to be found in the liver which are not common to most organs which may play an important rôle in preventing inflammations from gaining a foothold? Certainly the most important factor is the presence of a very highly developed and healthy system of endothelial cells. But it is not alone the mesenchymal portion of the liver that is bound up with this protective mechanism, it is definitely supported by the integrity of the liver cells and a very rich blood supply. But after all, this protection has its limits: we see this in the occasional colon infection extending out from the terminal bile ducts, in the metastatic abscesses following suppurative phlebitis of the portal vein, in the amebic and actinomyces infections, and also in tuberculosis, syphilis, typhoid and others, and lastly, as we have attempted to point out in this paper, we see this occasionally in streptococcus infection.

We have presented the lesions found in five different livers. Two of the three acute cases showed a rather acute inflammatory reaction with degeneration, necrosis of liver cells and an active cellular infiltration in which streptococci were demonstrable within the lesions.

The remaining two livers contain very unusual lesions which may be considered distinctly puzzling. One of these is probably a variety of a chronic lesion due to the immediate presence of streptococci within the liver, whereas the other may perhaps be considered a healed type of lesion due to a similar organism.

These five cases, two of them certainly and perhaps all, due to the immediate presence and action of streptococci, are presented for the purpose of calling attention to these pathological processes which might be overlooked or misinterpreted.

In our short series of experiments we have attempted — with certain reservations — to correlate lesions which may be produced in animals with lesions found in man. Lastly, in regard to the survival period of streptococci within the animal liver, considerable



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5. The last point that is considered is the histological and gross changes which one may find in the healed stage of these acute and chronic inflammatory lesions.

6. The second part of the paper is devoted to the results of experimental work. A streptococcus obtained from an early case of scarlet fever, was injected free of toxin into one of the radicles of the portal veins of both guinea pigs and rabbits. The animals were killed at varying intervals, and the lesions produced, together with the results of bacteriological studies, are fully described and compared with the lesions seen in human cases.

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## DESCRIPTION OF PLATES

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### PLATE 55

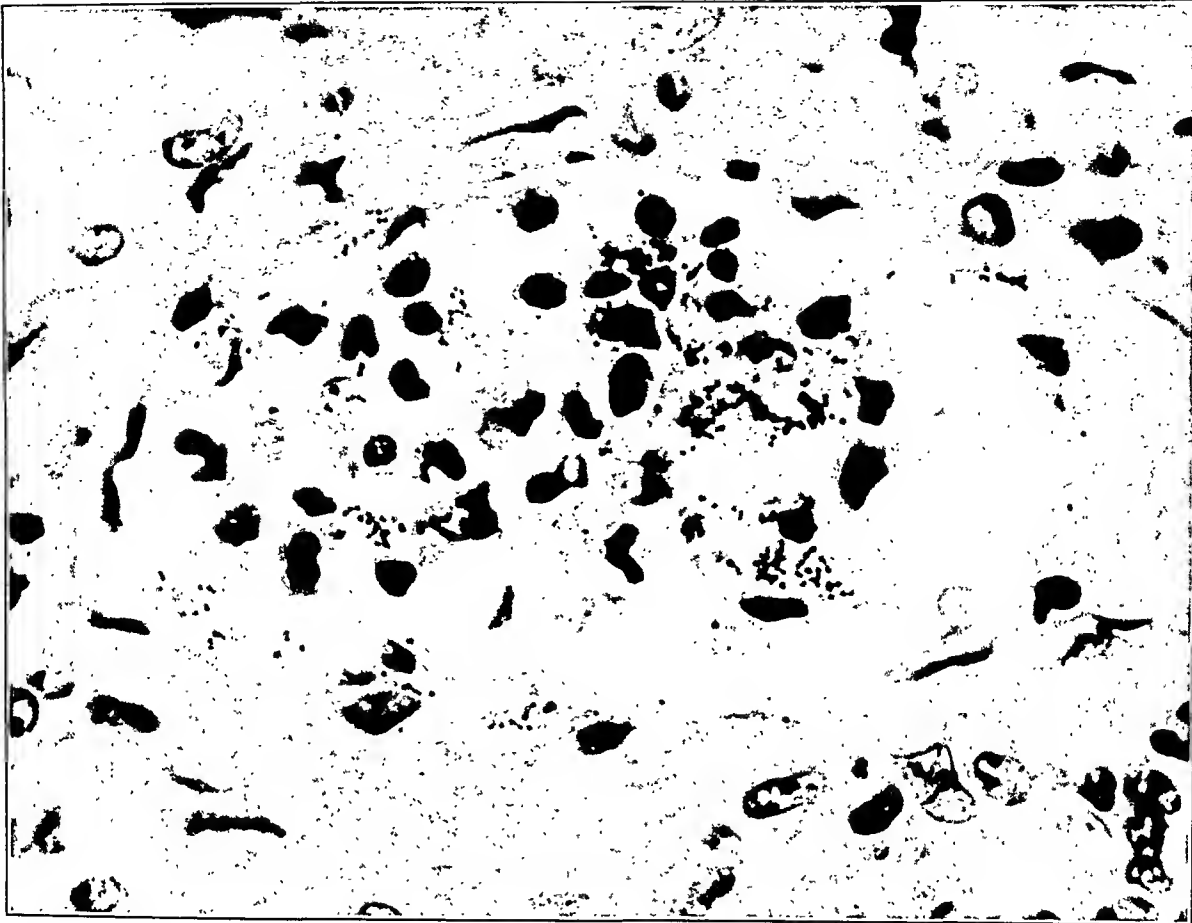
FIG. 1. Case 1. Masses of streptococci, largely in endothelial leucocytes, within a portal vein.  $\times 1000$ .

FIG. 2. Case 1. The sinusoids of the liver contain numerous streptococci of which many are included in the lining endothelial cells and in endothelial leucocytes filling the vessels.  $\times 1000$ .

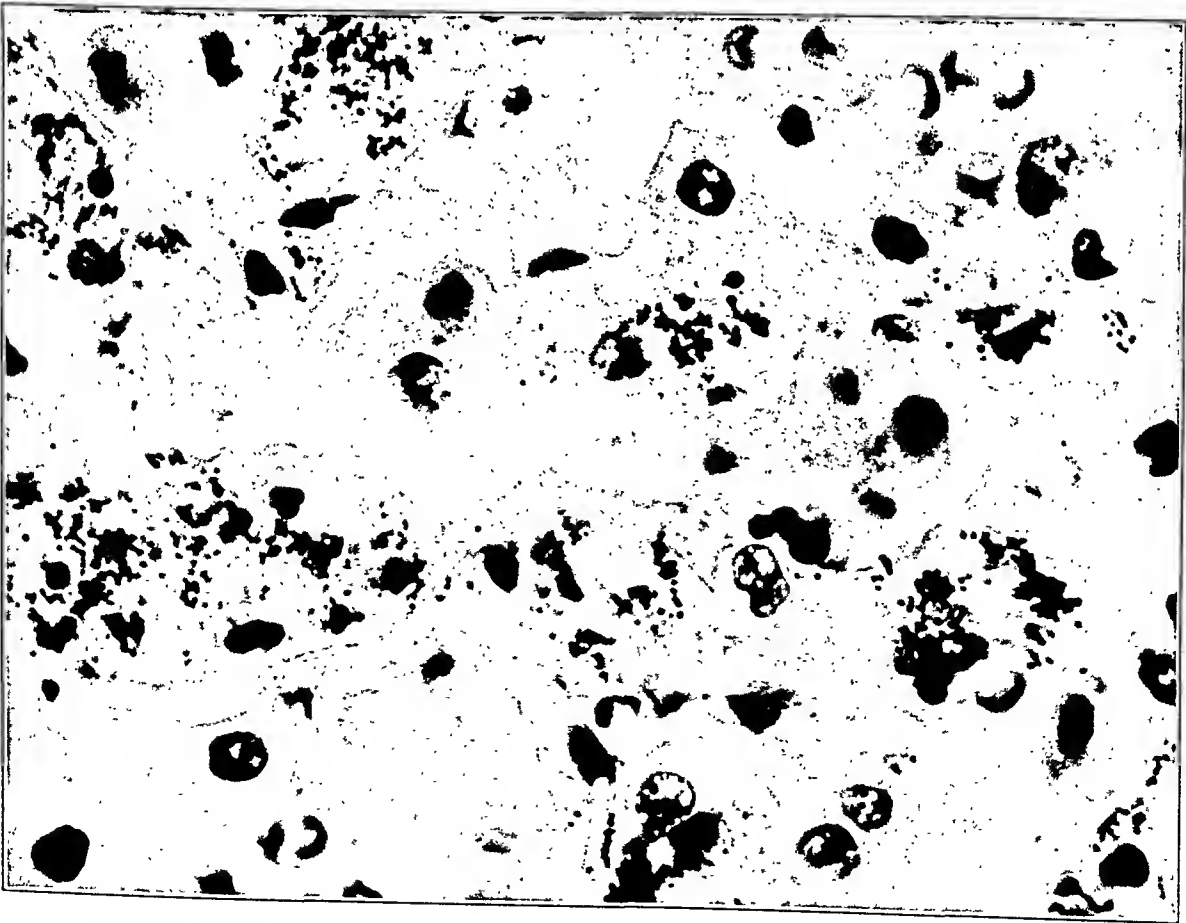
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PLATE 56

- FIG. 3. Case 1. An area in a lobule where the necrotic liver cells have to a large extent disappeared. Streptococci are still persistent in moderate numbers, largely in endothelial leucocytes.  $\times 1000$ .
- FIG. 4. Case 2. The edge of one of the two areas of necrosis involving many lobules. All of the liver cells have been killed and are being removed by the action of leucocytes. Only the bile ducts and stroma persist. The adjoining liver tissue is uninjured.  $\times 40$ .



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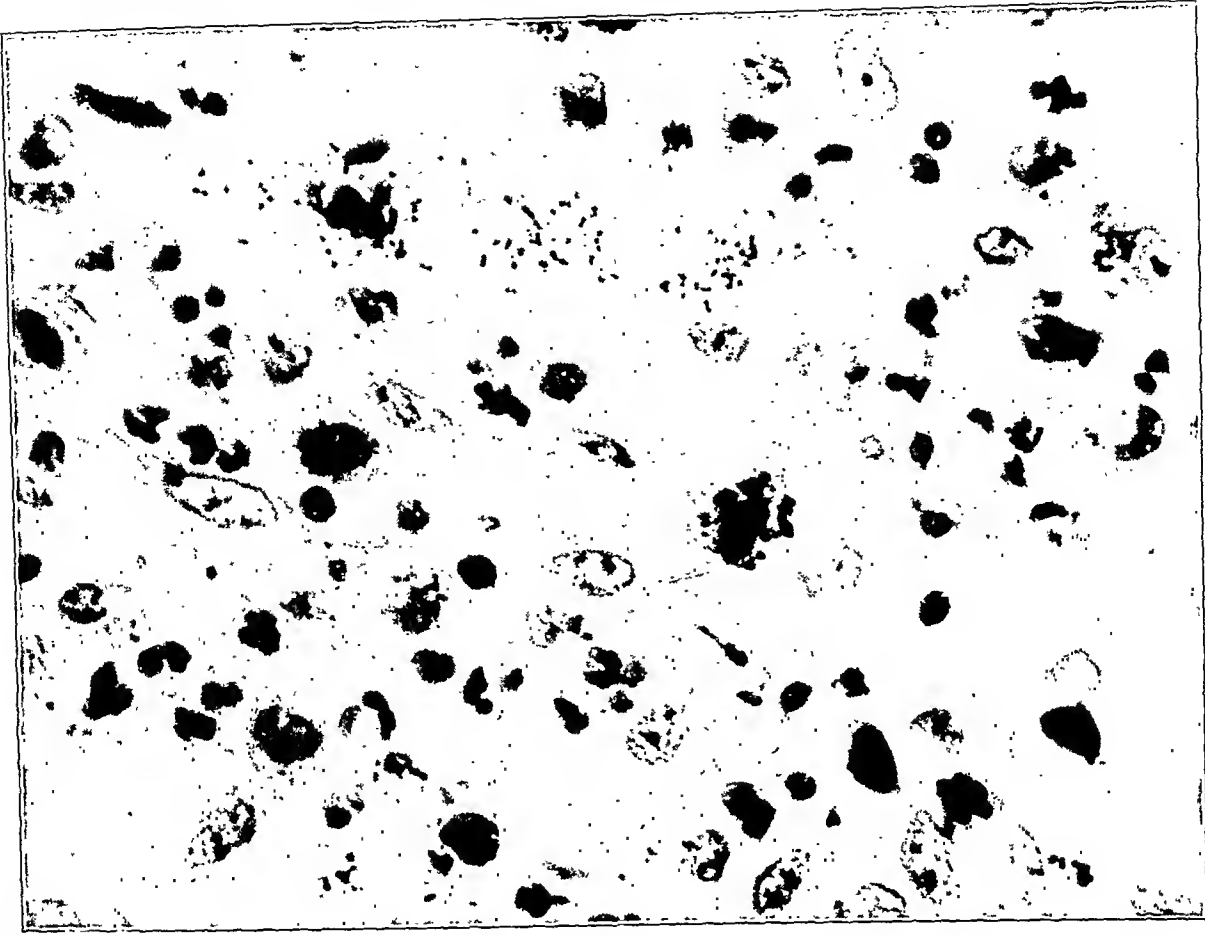


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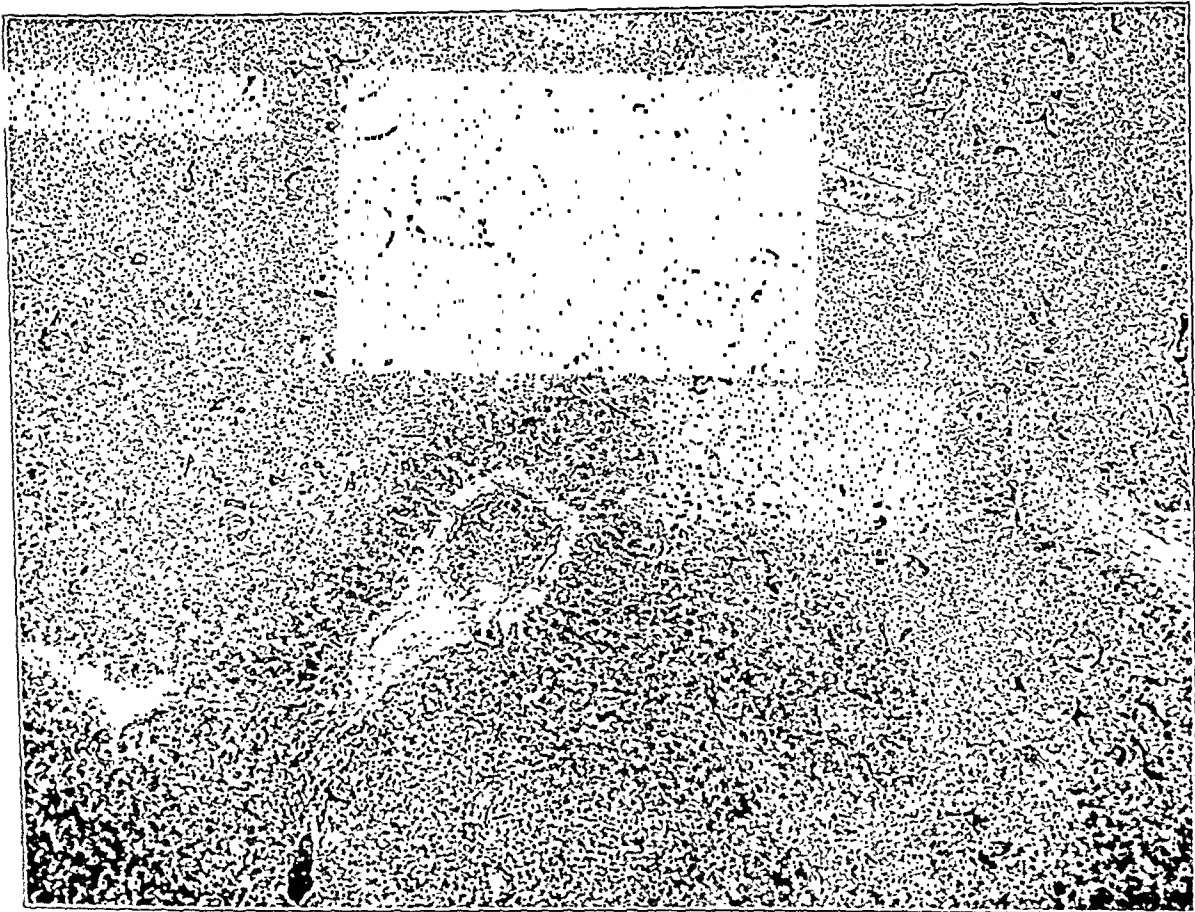
PLATE 57

FIG. 5. Case 3. The liver cells are necrotic in the peripheries of the lobules, presenting in places a zonal arrangement.  $\times 60$ .

FIG. 6. Case 3. A high power view of a small area in the necrotic zone. The nuclei of the liver cells have mostly disappeared. The sinusoids contain large clumps of streptococci.  $\times 1000$ .



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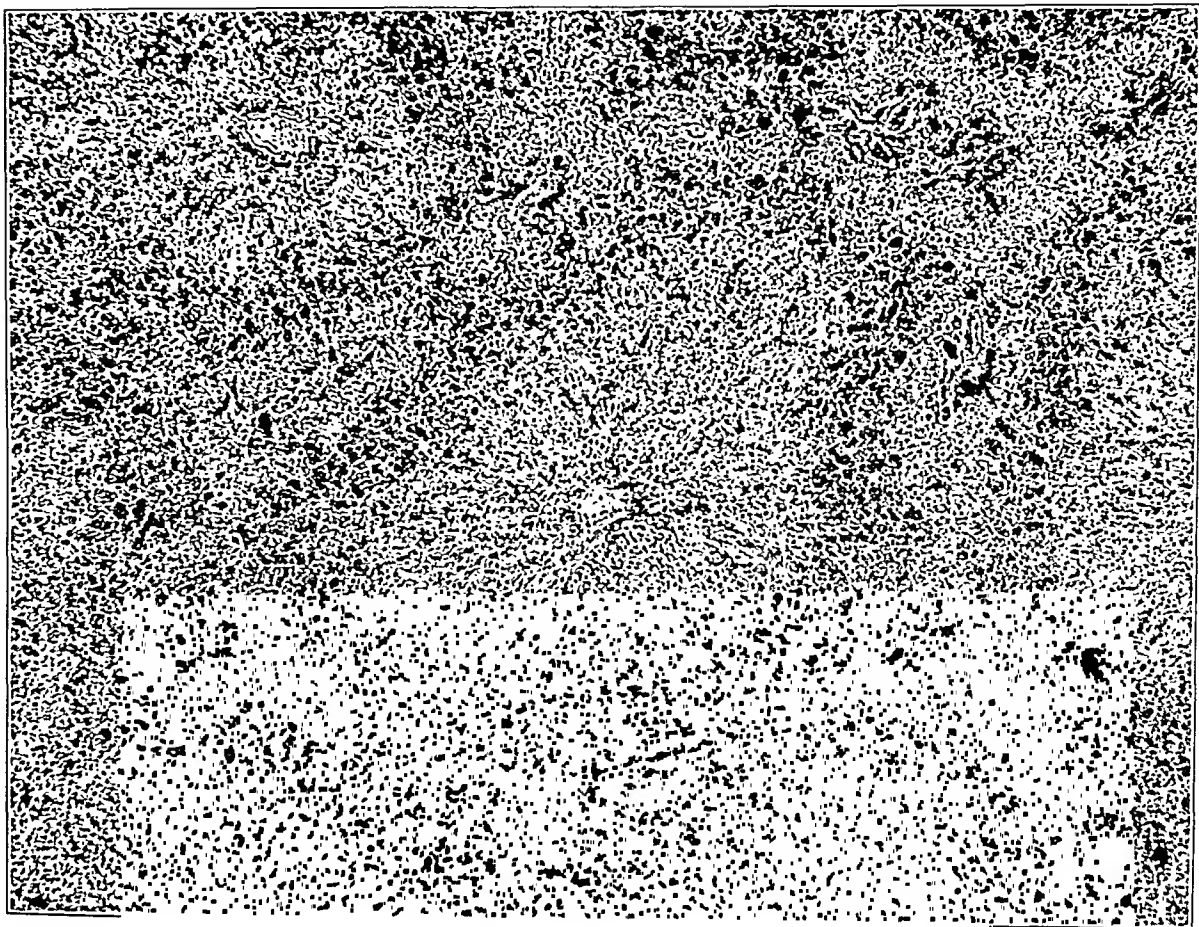
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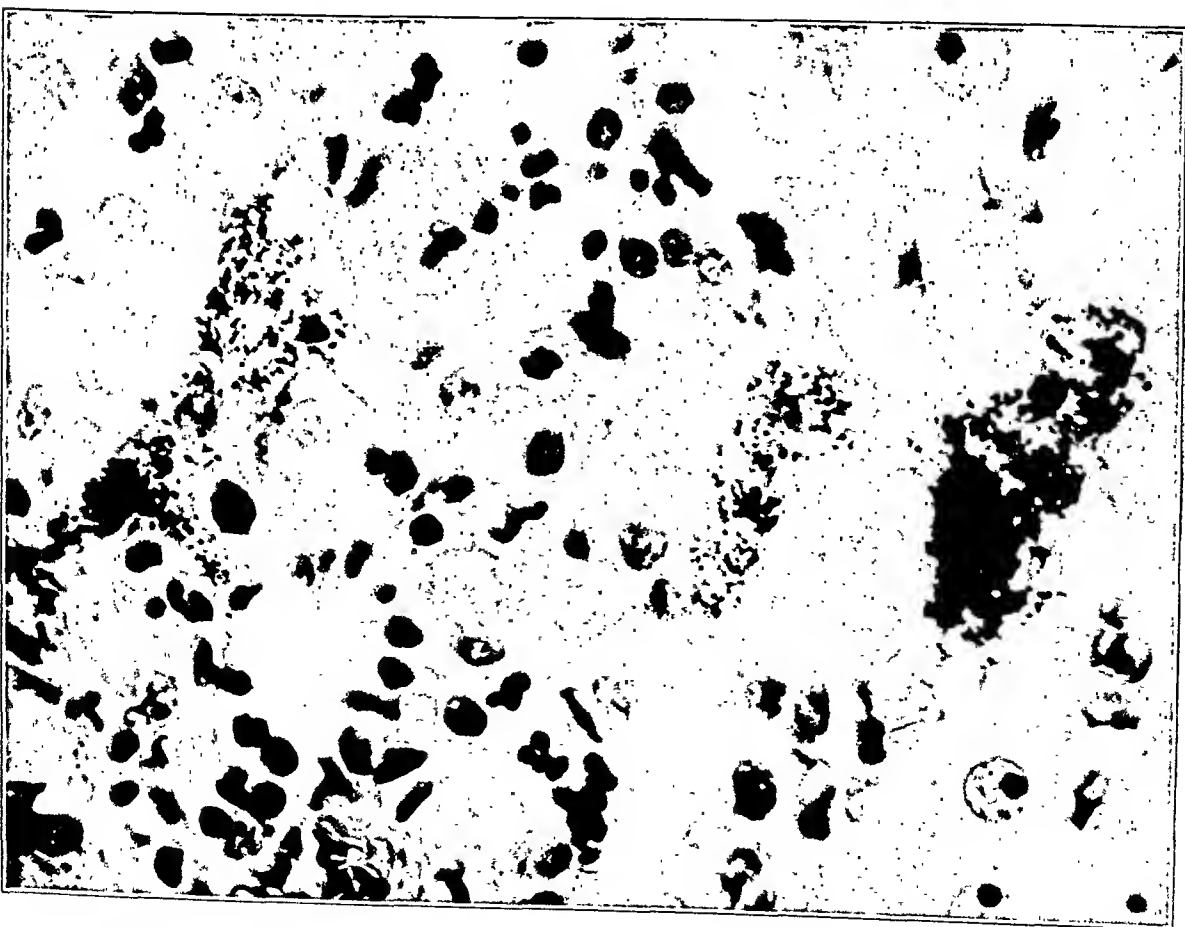
PLATE 58

FIG. 7. Case 4. A low power view showing the extensive formation of bile ducts. An occasional portal area can be made out.  $\times 50$ .

FIG. 8. Case 4. Marked formation of bile ducts. Only small islands of liver cells are present.  $\times 60$ .



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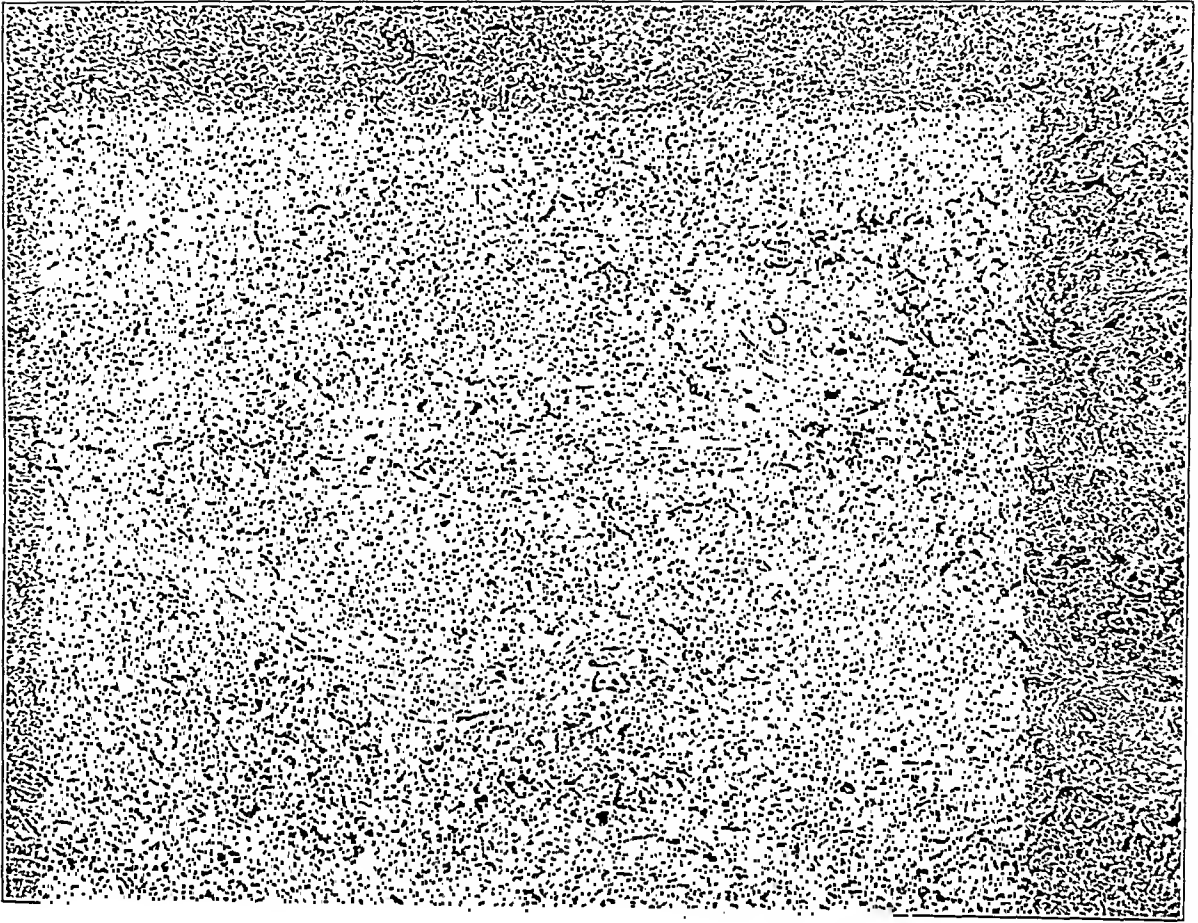


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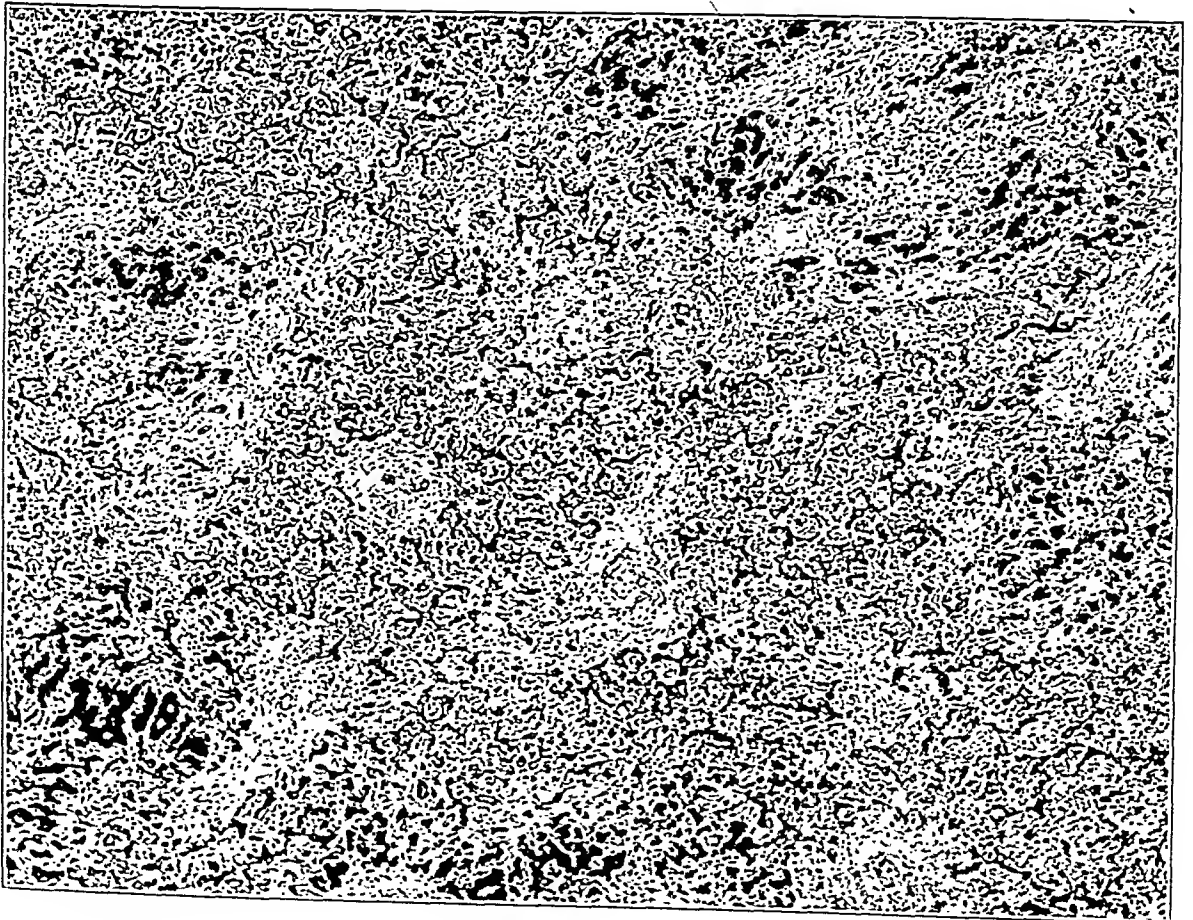
PLATE 59

FIG. 9. Case 4. High power view of network of bile ducts. The stroma is fairly abundant.  $\times 500$ .

FIG. 10. Case 4. Masses of streptococci mostly contained within endothelial cells lining the sinusoids.  $\times 1000$ .

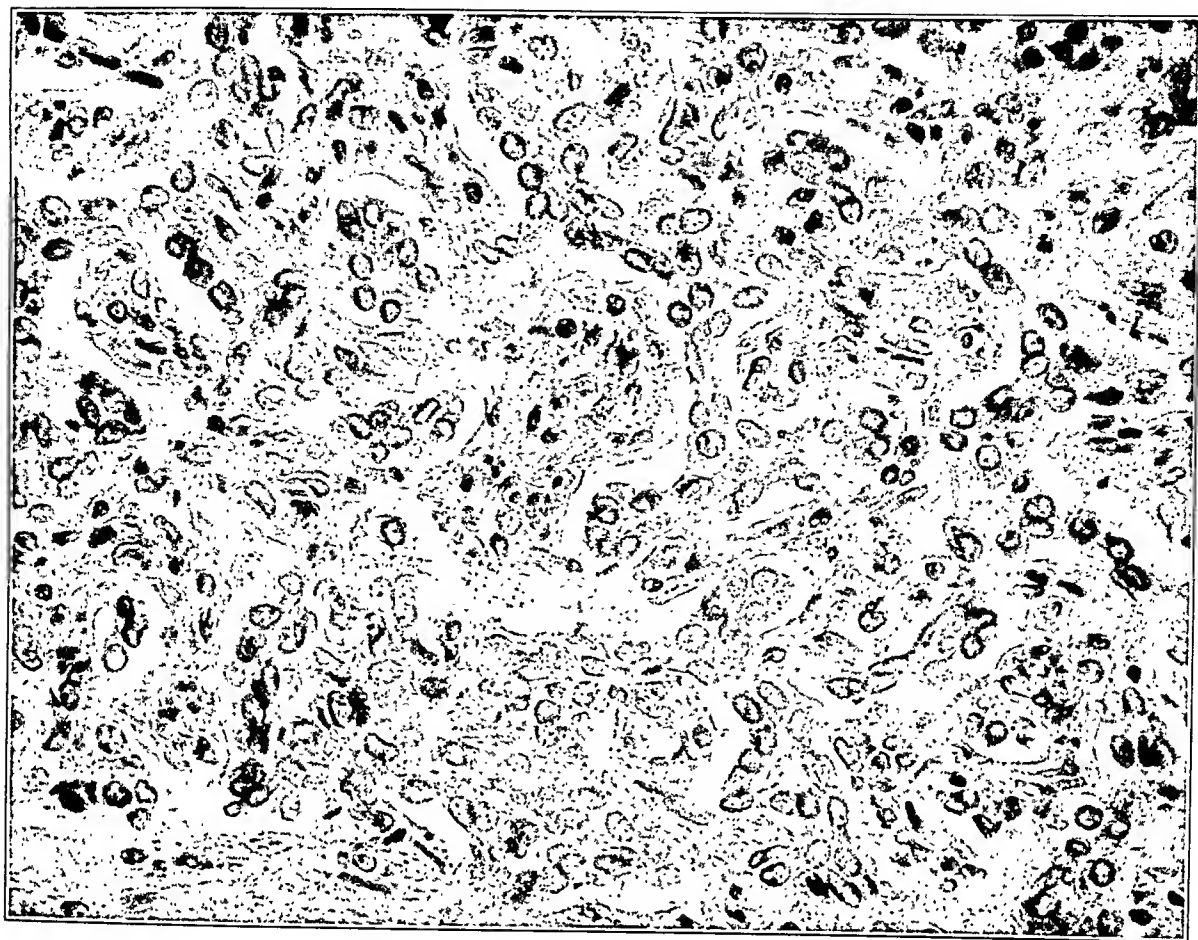


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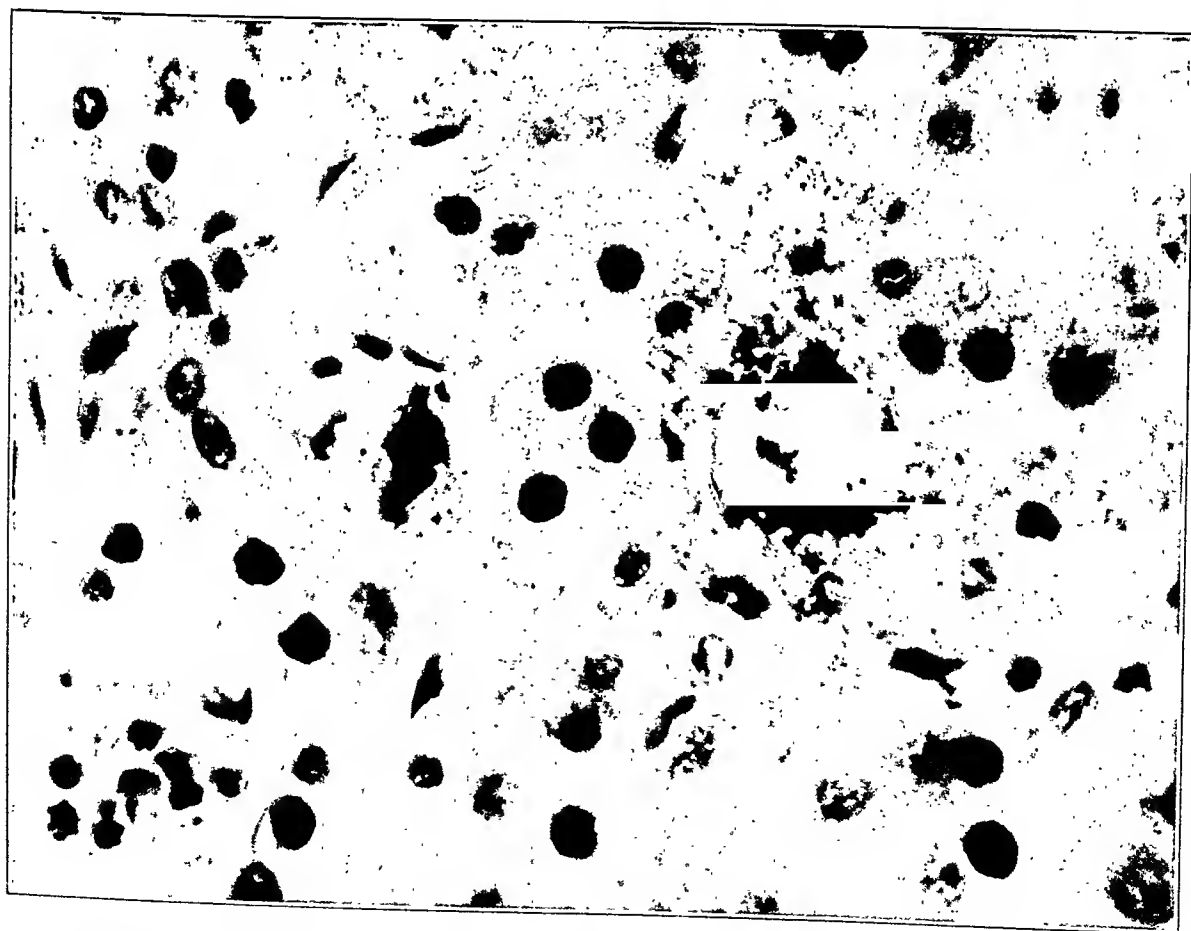


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*American Medical Association* of February 2, 1929, the editor cites the results of Flinn and VonGlahn to illustrate his remarks concerning the difficulties of animal experimentation. Admitting this report to be a "categorical denial" of the results of Mallory, he accepts, as proved, several statements which the authors hope to show are incorrect: first, the general assertion that copper and its compounds do not cause the deposition of pigment in the livers of laboratory animals; second, that the amount of copper in the tissues in experimental hemochromatosis is about the same as that in healthy tissues; third, that pigment cirrhosis can be produced in rabbits with sodium acetate; and finally, that experimental pigment cirrhosis in rabbits is probably caused by a diet top-heavy with carrots.

Polson,<sup>5</sup> working in England, has since published results which agree in general with the conclusions of Flinn and VonGlahn. He reported that copper acetate failed to produce a higher incidence of pigment cirrhosis in the liver than is found in normal controls when all of the rabbits are fed on cabbage, oats, bran and thirds. In a series of control rabbits fed on a diet containing mangel-wurzels and turnips, there was an accumulation of "hemofuscin in the livers of 88 per cent of the animals."

During the past two years German investigators have been very active in studying the "copper problem." Herkel,<sup>6</sup> in May, 1930, published a general review dealing with the biology and pathology of copper poisoning, including some experimental work. He has made a very complete review of the literature on experimental copper poisoning and the reader is referred to this article for a full account of the literature.

According to Herkel some of the earlier experiments were conducted by German and French workers about the middle of the 19th century. Among the earlier German investigators Ellenberger and Hofmeister,<sup>7</sup> 1883, obtained marked degenerative changes and deposition of pigment in the livers of sheep due to feeding copper sulphate. Filehne,<sup>8</sup> 1896, fed rabbits copper sodium-acid-tartrate and obtained fatty degeneration of the liver, connective tissue and bile-duct proliferation, together with a flaky pigmentation of the liver cells. A dog fed for two months on copper stearate gave a similar picture in the liver. In the same year Baum and Seeliger<sup>9</sup> obtained very definite changes in the livers of dogs, sheep, goats and cats (22 animals in all) following the feeding of cuprohemol, copper sulphate,

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## EXPERIMENTAL HEPATIC PIGMENTATION AND CIRRHOSIS\*

### I. DOES COPPER POISONING PRODUCE PIGMENTATION AND CIRRHOSIS OF THE LIVER?

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It is now ten years since Mallory, Parker and Nye<sup>1</sup> demonstrated the presence of pigmentation and cirrhosis of the liver in rabbits following the administration of copper salts in the feed. These authors called attention to the close similarity of the experimental lesions thus produced to those of hemochromatosis in man. Hall and Butt,<sup>2</sup> in 1928, repeated and extended the work of Mallory and his associates, obtaining pigmentation of the liver with early cirrhosis by feeding copper acetate and by injecting a weak solution of the same salt subcutaneously. These investigators found, on chemical analyses of the rabbits' livers, the presence of large quantities of copper in amounts roughly proportional to the various degrees of pigmentation.

In January, 1929, Flinn and VonGlahn<sup>3</sup> published the results of an investigation in which some of the earlier experiments of Mallory and his associates<sup>1</sup> were repeated and other experiments of their own added. These authors concluded that neither copper nor its compounds cause the deposition of pigment in the livers of rabbits, guinea pigs or rats, and neither do they produce cirrhosis. They also stated that a diet consisting exclusively of carrots will produce pigmentation of the liver in rabbits "in every way identical with that ascribed to copper." In an editorial<sup>4</sup> in *The Journal of the*

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grape vineyards of that region are treated with copper salts to prevent the growth of mildew and other fungi. Nine animals were given 200 mg. of copper acetate daily by mouth, while three others were given similar quantities of zinc acetate. One animal of the copper group died early of infection, the others were fed for from 61 to 249 days. The copper content of the livers varied from 52 to 170 mg. per kilogram of liver tissue, an average of 85 mg. Six normal rabbit livers were analyzed and yielded 6 mg. per kilogram of liver. There was considerable pigment deposited in the liver cells but in only two cases was pigment found in the Kupffer cells. The pigment did not stain selectively with fuchsin. There was slight increase in connective tissue but no true cirrhosis. The zinc-fed animals also developed moderate pigmentation of the liver cells. These authors failed to find the complex picture of early hemochromatosis comparable with that presented by Mallory, but on the other hand, they would not agree with the conclusions of Flinn and VonGlahn. They believe that copper alone is not responsible for the changes in the liver, but that some unknown factor plays a rôle.

Adrianoff and Ansbacher,<sup>12</sup> 1930, reported that cirrhosis developed in three out of four rats that were given copper. The presence or absence of pigmentation was not mentioned.

It is obvious, from a consideration of the wide divergence of results obtained by these various workers, that a reinvestigation under very exact and well controlled conditions of the whole question of copper poisoning in relation to hepatic pigmentation and cirrhosis, is desirable. Copper administered in sufficient quantities either does, or does not, produce pigmentation and cirrhosis of the liver in rabbits. The present investigation was undertaken for the purpose of answering this question. Whether or not the feeding of carrots, cabbage and other substances produces pigmentation, will be considered in another study.

## METHODS

In view of the conflicting results that have been obtained in the studies just cited, it was realized that exact methods and carefully controlled experiments would be necessary, if results of value were to be obtained. Rabbits of good stock were used throughout, since these animals are more sensitive to hepatic poisons than are rats or guinea pigs. The rabbits used were kept in individual, large,

copper acetate and copper oleate for periods of time up to one year. Besides the changes in the liver described by Filehne<sup>8</sup> these authors speak of the presence of an iron-containing pigment, hemosiderin, and an iron-free pigment which they call hematoidin. The pigment was found especially in the parenchyma cells. Similar changes are described as occurring in the kidneys. On chemical analysis of the livers a copper content was found as high as 0.2 per cent (200 mg. copper for 100 gm. liver substance).

More than a dozen of the earlier investigators cited by Herkel<sup>6</sup> obtained negative results in their copper-feeding experiments. Most of these studies, however, were not so carefully conducted or so well controlled as were those just cited. In several cases the duration of the experiments, or the dosage of copper employed, was not sufficient to produce pigmentation or cirrhosis.

Herkel's experiments are as follows: twelve rabbits were given daily 25 to 50 mg. of different copper salts (sodium tartrate, stearate and chloride) over periods of 8 to 154 days. Another group of five rabbits was given various combinations of the above copper salts combined with iron salts. These animals when killed showed in some cases a dark greenish brown color of the liver, otherwise they were normal. Histological studies showed rich deposits of round or granular pigment in the liver cells such as may be observed in the rabbit's liver under physiological conditions. The pigment was diffusely distributed in the liver cells. Only one animal showed connective tissue proliferation and this was not typical of cirrhosis. In general, their results agree with those of Flinn and VonGlahn and of Polson.

Eight rats were fed various copper salts (25 to 50 mg. daily for 42 to 112 days) as in the previous experiment, and eleven others were fed a combination of copper and zinc salts (25 mg. of each daily for 70 to 77 days). The results of the rat experiments were negative.

Chemical analyses of the rabbit and rat livers showed fairly large amounts of copper stored in the liver tissues. Quantities of copper up to 0.1 per cent failed to produce cirrhosis.

In a report from Aschoff's laboratory by Oshima and Siebert,<sup>10</sup> 1930, the question of experimental copper poisoning is reviewed and the results of their experiments recorded. This work was undertaken, according to Schönheimer,<sup>11</sup> because of the discovery of ten to twelve cases of hemochromatosis in Freiburg within the period of about a year, and further on account of the fact that the extensive

of copper with each gram of food. The rabbits were weighed at five-day intervals.

When a copper-fed rabbit was killed or died, its litter-mate control was also sacrificed and the carcass carefully examined. Since the intestinal contents of the rabbit constitute a variable but considerable portion of the body weight, the weight of the entire gastrointestinal tract and its contents was subtracted from the gross body weight. The "net" body weight so obtained was used for comparison. The liver in each case was carefully removed, and if both the copper-fed and the control animals had been killed, each was drained of gross blood. If the copper-fed rabbit had been dead for some hours, no attempt was made to free the control liver of blood. The liver was weighed and representative pieces of tissue taken for histological examination. The remainder of the organ was again weighed, dried at 105° C to constant weight, and preserved for biochemical analyses. Many of the animals showed some coccidial infestation. If one of a pair was infested, the litter-mate was usually also infested. In any case this factor appeared to have little influence on the final results. All the other viscera besides the liver were examined grossly, and histological studies were made in a few cases. No changes of consequence were found outside of the liver.

The amount of copper in the livers was determined by the micro-method of Elvehjem and Lindow.<sup>13</sup> This method was checked in a number of instances by the standard macromethod.

Blocks of liver tissue from each animal were fixed in 95 per cent alcohol and in formalin. In a few cases blocks from other organs were also fixed in these solutions. The alcohol-fixed material was kept in reserve. Sections were stained with hematoxylin and eosin; also with potassium ferrocyanide and hydrochloric acid followed by a 0.5 per cent solution of basic fuchsin in 50 per cent alcohol. This stain causes hemosiderin granules to assume a dark blue color (Berlin blue reaction for iron), while the basic fuchsin stains hemofuscin deep red in either the presence or absence of hemosiderin. Another method, recommended by Mallory,<sup>1</sup> was found very useful, especially if photomicrographs showing the hemofuscin were to be made. This method consists in staining the sections in alum hematoxylin, followed by a mixture of 1 part of strong yellow ammonium sulphid to 3 parts of 95 per cent alcohol for 1 to 2 hours. The latter operation should be carried out in a glass staining dish with a tightly fitting

roomy, well protected outdoor hutches, with twelve square feet of floor space, placed on a hillside with a southeast exposure. The temperature range of 55° to 70° F was not exceeded at any time.

An attempt was made to keep the conditions of the copper-fed group and the control group exactly the same, except that one group received copper in the form of normal copper acetate added to their food. This desirable state of affairs was nearly attained. The one difference, as we shall see, rested in the loss of appetite of the copper-fed rabbits and their failure to eat as much food as did the controls.

All of the rabbits were placed on a special control diet when they were exactly 85 days of age. This diet consisted of a mixture of 75 parts of whole ground alfalfa and 25 parts of whole ground barley. The control rabbits continued to receive this diet until the end of the experiment, while the copper group commenced to receive the copper diet when exactly 90 days of age. The copper diet was the same as the control diet, except that it contained 2 mg. of copper per gram of food in the form of normal copper acetate. The copper-containing food was prepared by the addition of 10 cc. of 63 per cent copper acetate solution to each kilo of the control diet. Actually the copper solution was added to a small portion of the food, which was then dried at 50° C, after which it was intimately mixed with the remainder. These diets, together with tap water, were allowed the respective groups *ad libitum*. For every rabbit which received the copper diet, a *litter-mate of the same sex* was fed the similar but non-copper-containing control diet. This is generally looked upon as the optimal method of control in animal feeding experiments.

The experiments were not continued for any definite length of time. Some of the copper-fed rabbits died and their controls were killed after the experiment had continued for 21 to 60 days. All but five were left on the copper diet until they succumbed, presumably from copper poisoning. It is reasonable to suppose that these animals died of copper poisoning because not a single control rabbit died before being sacrificed at the time of death of its copper-fed litter-mate. All of the control animals were in excellent condition when killed. The food intake was measured daily. Although there was some unavoidable scattering, the diets were both very light, and we believe that the food intake figures are reasonably accurate. For present purposes the copper intake of the *control* rabbits was so small that it is negligible, while the copper-fed rabbits received 2 mg.

TABLE I  
Complete Data on Copper-Feeding Experiment

No.	Group	Sex	Variety	Litter No.	Days on diet	Gross body wt.	Net body wt.	Liver wt. (wet)	Liver per kg. (wet)	Copper liver as percent- age control	Liver (dry)	Liver per kg. (dry)	Copper ingested during exp.	Copper per 100 gm. (wet) liver	Pigments		Cirrhosis	Necrosis
															Hemo- fuscin	Hemo- siderin		
*80	Control	F	Flem. Cross	11	21	Kg. 1.85	Kg. 1.36	gm. 65	gm. 47.8	per cent	gm. 16.8	gm. 12.3	gm. ..	mg. 0.01	-	-	-	-
79	Copper	F	"	11	21	1.05	0.75	31	41.3	88.7	6.9	9.2	2.68	133.00	++	+	-	-
*87	Control	F	Albino	14	24	1.59	1.25	38	30.8	..	10.3	8.2	..	0.00	-	-	-	-
88	Copper	F	"	14	24	1.42	1.11	42	37.8	122.5	8.5	7.7	3.96	70.80	-	-	-	-
*85	Control	F	"	13	29	1.80	1.42	61	43.0	..	15.2	10.7	..	0.00	-	-	-	-
86	Copper	F	"	13	29	1.42	1.01	49	48.4	112.6	9.8	9.7	4.28	9.68	++	-	-	++
*89	Control	F	"	15	29	1.88	1.47	65	44.3	..	14.3	9.7	..	0.00	-	-	-	-
90	Copper	F	"	15	29	1.41	1.14	37	32.4	73.2	7.2	6.3	4.01	115.00	++	-	-	-
*91	Control	F	"	16	29	1.43	1.11	51	45.9	..	10.8	9.8	..	0.00	-	-	-	++
92	Copper	F	"	16	29	1.24	0.93	34	36.6	79.7	7.0	7.5	4.56	128.00	++	-	-	++
*82	Control	F	Flem. Cross	11	30	2.18	1.70	76	44.7	..	18.5	10.9	..	0.00	++	-	-	-
81	Copper	F	"	11	30	1.09	0.77	33	42.8	95.8	6.4	8.3	6.32	0.00	++	+	-	-
*47	Control	M	Albino	6	40	1.60	1.05	62	59.0	..	16.0	15.3	..	128.40	++	-	++	++
49	Copper	M	"	6	40	1.76	1.24	29	23.4	39.7	6.3	5.1	11.24	216.00	++	-	++	-
*76	Control	F	Flem. Cross	10	40	2.69	2.24	60	26.8	..	10.9	4.5	..	0.00	-	-	-	-
77	Copper	F	"	10	40	1.28	0.97	23	23.7	88.5	4.0	3.1	5.73	77.40	++	-	-	-
*50	Control	M	Albino	6	44	2.57	1.87	101	54.0	..	24.4	13.0	..	0.00	++	-	-	-
48	Copper	M	"	6	44	1.77	1.18	50	42.4	78.4	11.4	9.7	13.62	90.70	++	-	-	-
*71	Control	F	Amer. Blue	8	45	2.39	1.83	97	53.0	..	25.3	10.6	..	0.01	-	-	-	-
72	Copper	F	"	8	45	1.52	1.15	28	24.3	45.9	5.7	5.0	7.38	171.80	++	+	-	++

cover to prevent loss of the ammonia. After thorough washing in water, sections are stained in 0.5 per cent basic fuchsin as described above for 20 minutes. Destain in 95 per cent alcohol and dehydrate, running through absolute alcohol, xylol, and mounting in xylol colophonium. The result is a beautiful differentiation showing nuclei blue, *hemofuscin* dark red, *hemosiderin* black (Fig. 5). Mallory's connective tissue stain (Fig. 2), also hematoxylin and Van Gieson stains, were used to bring out the connective tissue in the cirrhotic livers.

## RESULTS

The results of our experiments are presented in concise form in Table I. The data on the copper-fed animal and its litter-mate control in each instance are placed side by side for the purpose of easy comparison. The duration of the experiments was from 21 to 105 days. In the shorter periods (21 to 60 days), the duration of the feeding was determined by the death of the copper-fed animal, while in no case did a control animal die. Of the twenty-one animals on the copper diet, sixteen died of copper poisoning. Marked changes in the liver in the way of pigmentation or fibrosis are hardly to be expected to develop in such short periods of time. Yet it will be seen that Rabbits 79, 86, 90, and 92 — animals that were on the copper diet only 21 to 29 days — showed moderate pigmentation in the form of "hemofuscin," while Rabbit 79 also developed a moderate amount of iron-containing pigment. While some pigment granules are found in the hepatic cells, by far the greater quantity occur in large Kupffer cells which have apparently agglomerated to form multinuclear giant cells. The cytoplasm of these cells is loaded with yellowish brown granules that stain red with basic fuchsin.

In each of the above animals the "net" body weight is less than that of the litter-mate control. In Rabbit 90, the difference is only slight, while in Rabbit 79 there is almost 50 per cent decrease. Likewise there is a decrease in the weight of the livers in the copper-fed animals ranging from 12 to 27 per cent, except in Rabbit 86, which shows a 12 per cent increase in weight over its litter-mate control. Study of the quantities of copper ingested by these four animals shows 2.68 gm. for Rabbit 79, while the other three consumed 4.28, 4.01 and 4.56 gm. respectively. It will be seen that the intake of copper is quite proportional to the length of time that the animals

were on the copper diet. The amounts of copper stored in the livers are somewhat more variable, Rabbit 79 having the lowest intake, yielded 133 mg. of copper per 100 gm. of wet liver tissue. Corresponding to this is a considerable deposit of hemofuscin and hemosiderin in the liver. For some unknown reason Rabbit 86 yielded only 9.68 mg. of copper in spite of a relatively high intake. Notwithstanding this low copper content, a moderate amount of hemofuscin was found in the liver. The other two animals showed on analysis 115 and 128 mg. of copper respectively.

Rabbit 88 is the only animal in this group that failed to show pigmentation. It was fed for 24 days, ingested 3.96 gm. of copper and stored 70.8 mg. in each 100 gm. of liver tissue. The liver was large (122.5 per cent of control), mottled in appearance and microscopically showed well marked congestion and central necrosis. The histological picture is that of a well advanced chronic passive congestion.

Of the sixteen animals fed copper acetate for 30 days or more, ten showed cirrhosis and thirteen showed pigmentation. Of the five animals in the copper-fed group on the diet for over 60 days, all showed pigmentation to an extreme degree, while four animals presented a well marked cirrhosis.

The copper ingested by the rabbits fed for 30 days or more ranged from 5.73 gm. to 19.60 gm. The latter amount was ingested by the 105-day animal. The amounts of copper ingested correspond roughly to the duration on the diet. The copper content of the various livers, however, is most interesting in relation to the presence of pigmentation and cirrhosis. In a general way, it may be stated that livers containing over 100 mg. of copper per 100 gm. of wet liver substance showed cirrhosis. There are only four exceptions to this in the animals fed for more than a month (Table II) and three of these presented necrosis of the liver tissue and at the same time heavy pigmentation. It is interesting to note that the copper-fed animals which developed necrosis failed to show increase in fibrous tissue.

Only two animals, Rabbits 13 and 64, out of the sixteen fed copper acetate for a month or more, failed to show either pigmentation or cirrhosis. The copper content of the livers in these animals was 53.4 and 86.5 mg. per 100 gm. of wet liver substance respectively. It would seem, therefore, that pigmentation fails to develop until approximately 75 to 100 mg. of copper are stored in each 100 gm. of liver tissue.





that sodium acetate was substituted for copper acetate. Littermates of the same sex were kept as controls. The animals were kept on the sodium acetate-containing diet for periods varying from 40 to 60 days. None of these animals showed either pigmentation or cirrhosis. The control animals were likewise negative as to liver changes.

### COMMENT

*Early Lesions in the Liver:* The earliest change seen in the rabbit's liver in chronic copper poisoning is a moderate enlargement of the Kupffer cells, which can be seen to contain many small golden yellow granules which stain red with basic fuchsin. Potassium ferrocyanide combined with a weak solution of hydrochloric acid fails to stain the granules. This pigment has been identified by Mallory as hemofuscin, and he believes it to be the same as the iron-free pigment described by von Recklinghausen<sup>14</sup> in hemochromatosis. Occasionally in copper poisoning, darker granules may be seen in the Kupffer cells and in the hepatic cells. This pigment is, no doubt, hemosiderin, since it gives the Berlin blue reaction, and it corresponds to the iron-containing pigment which is so abundant in hemochromatosis in man.

*Kupffer Giant Cells and Pigmentation:* In livers that show somewhat more advanced changes, the Kupffer cells are seen to be greatly enlarged and many have become detached and have moved towards the portal spaces. Here groups of six to eight cells have fused to form large multinuclear giant cells (Figs. 3 and 5). These large cells apparently form in response to the presence of the hemofuscin pigment, since the two are always associated together. In some cases considerable amounts of pigment, similar to that seen in the Kupffer cells, are found in the liver cells as well. In a general way, pigment seems to accumulate in the liver cells in preference to the Kupffer cells when the feeding experiments are of long duration, and the daily copper intake therefore reduced in amount. On the other hand, larger daily feedings of copper which produce death in 30 to 60 days tend to call forth the large Kupffer cells. As the pigment accumulates rapidly in the liver, the Kupffer cells, unable to cope with the situation, mobilize and fuse to form giant cells just as the macrophages fuse about bits of suture material or foreign bodies which it seems the individual cells are unable to dissolve.

In Table II, all of the copper-fed animals are grouped together in a simplified table showing only the more important factors. This gives a more concise picture of the positive findings in a form easily comparable. It shows especially well the relation of pigmentation and cirrhosis to the ingestion and storage of copper.

TABLE II

*Relation of Pigmentation and Cirrhosis to Copper Content*

No.	Days on diet	Ratio Cu to control livers	Copper ingested	Copper per 100 gm. (wet) liver	Hemofuscin	Hemo-siderin	Cirrhosis	Necrosis
		<i>per cent</i>	<i>gm.</i>	<i>mg.</i>				
79	21	88.7	2.63	133.00	++	++	-	-
88	24	122.5	3.96	70.80	-	-	-	++
86	29	112.6	4.28	9.68	++	-	-	-
90	29	73.2	4.01	115.00	++	-	-	+++
92	29	79.7	4.56	128.00	+++	-	-	+++
81	30	95.8	6.32	128.40	+++	+	+++	-
49	40	39.7	11.24	216.00	++++	-	++	-
77	40	88.5	5.73	77.40	++	-	-	+++
48	44	78.4	13.62	90.70	+++	-	-	-
72	45	45.9	7.38	171.80	++	+	++	-
65	50	124.4	8.96	190.50	+++	-	++	-
13	60	95.6	13.67	53.40	-	-	-	-
18	60	77.4	13.27	81.50	++	-	-	-
20	60	75.0	13.37	126.40	+++	+	-	-
64	60	110.0	8.93	86.50	-	-	-	-
67	60	140.0	13.93	129.00	+++	-	++	-
38	63	95.4	10.81	186.20	++++	-	++	-
46	69	80.6	14.20	198.40	++++	+	+++	-
84	70	70.0	11.25	237.00	++++	-	++	-
73	74	88.4	16.11	193.10	++++	-	++	-
39	105	89.8	19.60	188.00	++++	-	-	+

The question, raised by Flinn and VonGlahn<sup>3</sup> as to the production of pigmentation and cirrhosis by feeding sodium acetate, was also investigated. These authors suggest that the acetate radical is responsible for the liver changes where the acetate salt of copper is used in the feeding experiments. The photomicrograph (Fig. 3), which they show to illustrate the pigment found in the liver following sodium acetate feeding, is not at all convincing, since similar quantities of pigment may be seen in the liver cells of control rabbits, especially if the animals are relatively old. We have fed three rabbits on a diet similar to that of our copper-fed animals, except

## CONCLUSIONS

In consideration of the well controlled experiments here presented, in which seventeen out of twenty-one copper-fed animals showed pigmentation, many of them to a most extreme degree, and in view of the fact that nearly 50 per cent of this same group showed cirrhosis (this also being well marked and indisputable) we must conclude, in spite of the adverse reports by Flinn and VonGlahn,<sup>3</sup> Polson,<sup>5</sup> Herkel<sup>6</sup> and others, that copper poisoning in rabbits produces pigmentation and cirrhosis of the liver in a high percentage of cases if adequate doses are given. This conclusion is in accord with the original work of Mallory<sup>1</sup> and with the later investigation of Hall and Butt.<sup>2</sup>

We have found in most cases, at least under the conditions of our experiments, that 75 to 100 mg. of copper must be stored for every 100 gm. of wet liver tissue before pigmentation and cirrhosis result. The latter process apparently requires more time, and larger amounts of copper must be present in the liver tissue than are required to bring about pigmentation alone.

That large quantities of copper are stored in the liver of the copper-fed animals as compared with that found in the controls, is very evident on examination of Table I.

None of our animals received carrots, mangel-wurzels, turnips, cabbage or other plant substance rich in carotin, therefore the question of pigmentation due to these substances does not enter as a factor in the results presented here. The question of whether or not heavy feeding with carrots does produce pigmentation will be considered in a subsequent study.

The resemblance of the lesions described in this paper to those of early hemochromatosis in man is very striking.

## SUMMARY

1. The results of a study of copper poisoning in rabbits are presented in which the experiments were rigidly controlled. A litter-mate control of the same sex was fed a special control diet for each animal that received copper.

2. Twenty-one rabbits were fed on a diet containing 2 mg. of normal copper acetate in each gram of food. The copper salt was

In each of our animals in which pigmentation was present, multinuclear giant cells were the most prominent part of the picture, and contained most of the pigment. In a few cases many pigment granules were also found in the liver cells. In the former experiments on copper poisoning by Hall and Butt, in which the animals were fed over periods of 33 to 35 weeks, the greater part of the pigment was present in the liver cells, although a few of the livers contained large giant cells as well. In recent experiments by Butt,<sup>15</sup> in which rabbits were fed copper for more than a year, the pigment is diffusely distributed in the liver cells.

No attempt will be made in the present study to inquire into the nature of the so-called "hemofuscin" which occurs in copper poisoning except to state that Mallory believes, and the writers agree with him, that hemofuscin resulting from copper poisoning contains considerable quantities of copper. A number of investigators including Mallory,<sup>1</sup> Abbott,<sup>16</sup> and the writers, believe that hemofuscin also contains iron in bound form.

Only five of our animals showed any hemosiderin and this was in small amounts. In nearly every case in which this pigment occurred, it was found in both the liver cells and the Kupffer giant cells.

*Experimental Cirrhosis:* In the original experiments by Mallory, *et al.*,<sup>1</sup> three copper-fed animals out of twenty-two developed cirrhosis. The periods of time required on the copper diet were  $5\frac{1}{2}$ ,  $6\frac{1}{2}$ , and  $11\frac{1}{2}$  months respectively. Hall and Butt<sup>2</sup> obtained fibrosis of mild degree in a number of animals fed for periods of 8 to 9 months. The writers have obtained a cirrhosis in nine out of twenty-one copper-fed animals. If the five animals that were on the copper diet for a period less than thirty days be omitted, because of the short time, the incidence of cirrhosis is very high, amounting to 53 per cent. Furthermore, the cirrhosis is very definite, resembling human cirrhosis closely, as can be seen by referring to Figs. 2, 4 and 6. In the sections stained with hematoxylin and eosin, many giant cells loaded with yellow pigment granules may be seen scattered throughout the fibrous tissue (Figs. 4 and 6), while only occasional giant cells are seen elsewhere. This association of the pigment with the fibrous tissue, and the fact that the pigment precedes the development of the fibrosis, are presumptive proofs that the presence of the pigment stimulates the growth of connective tissue. A somewhat analogous condition may be found in the various pneumoconioses due to extraneous pigmentation of the lungs.

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## DESCRIPTION OF PLATES

### PLATE 60

- FIG. 1. Rabbit 84, fed copper acetate for 70 days. Photomicrograph of liver (low power) showing numerous Kupffer giant cells filled with hemofuscin. The pigment is somewhat more abundant about the periportal spaces. Basic fuchsin stain.
- FIG. 2. Rabbit 49, fed copper acetate for 40 days. Liver showing well developed cirrhosis with new fibrous tissue surrounding the lobules. Many pigment-containing giant cells in the connective tissue. Mallory's connective tissue stain.

added to the control diet, which consisted of 75 parts of whole ground alfalfa and 25 parts of whole ground barley.

3. The duration of the experiment was from 21 to 105 days.

4. Seventeen of the copper-fed animals showed pigmentation of the liver, mostly in the form of hemofuscin stored in Kupffer giant cells.

5. Nine of the twenty-one copper-fed animals showed cirrhosis of the liver.

6. Five of the animals, which failed to show cirrhosis, showed varying degrees of liver cell necrosis.

7. Three animals in which sodium acetate was substituted for the copper salt failed to show changes in their livers.

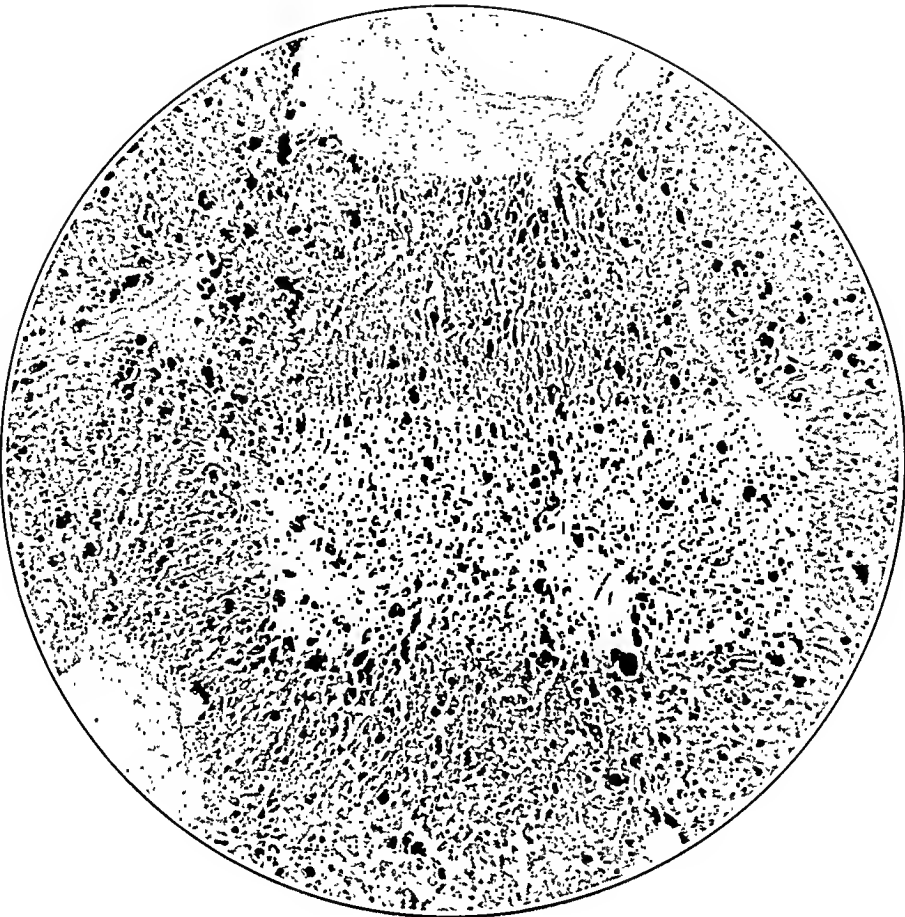
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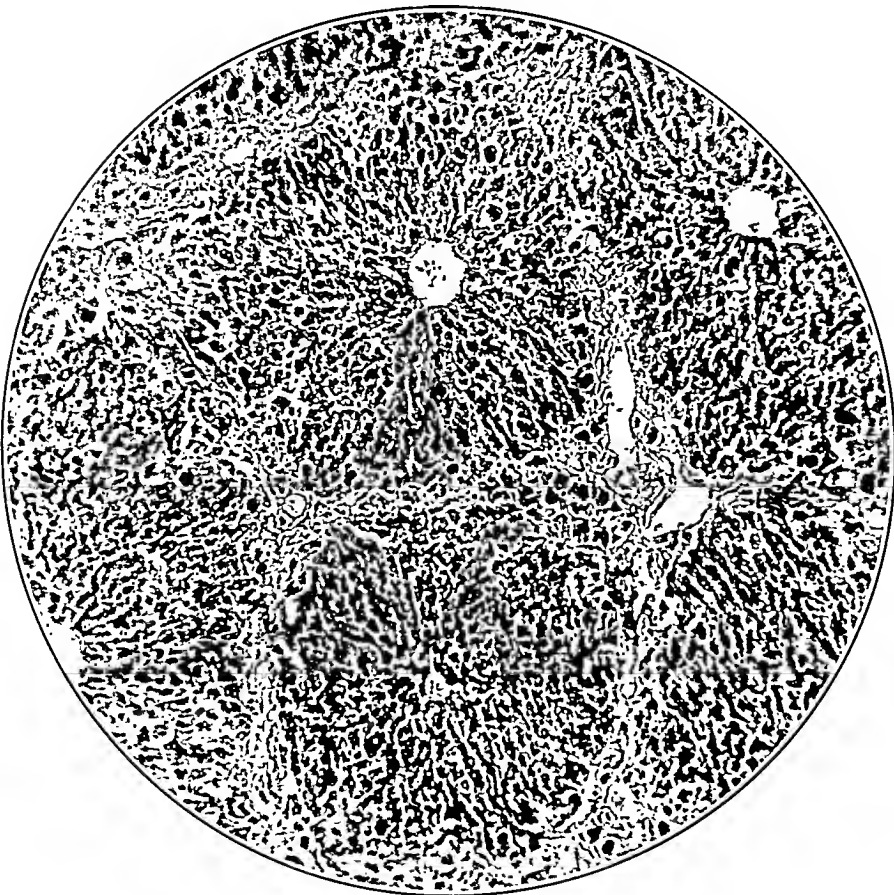
PLATE 61

FIG. 3. Rabbit 46, fed copper acetate for 69 days. High power photomicrograph of liver showing numerous Kupffer giant cells loaded with hemo-fuscin. Basic fuchsin stain.

FIG. 4. Rabbit 81, fed copper acetate for 30 days. Low power photomicrograph showing marked development of fibrous tissue in which many large pigment giant cells occur. Hematoxylin and eosin stain.



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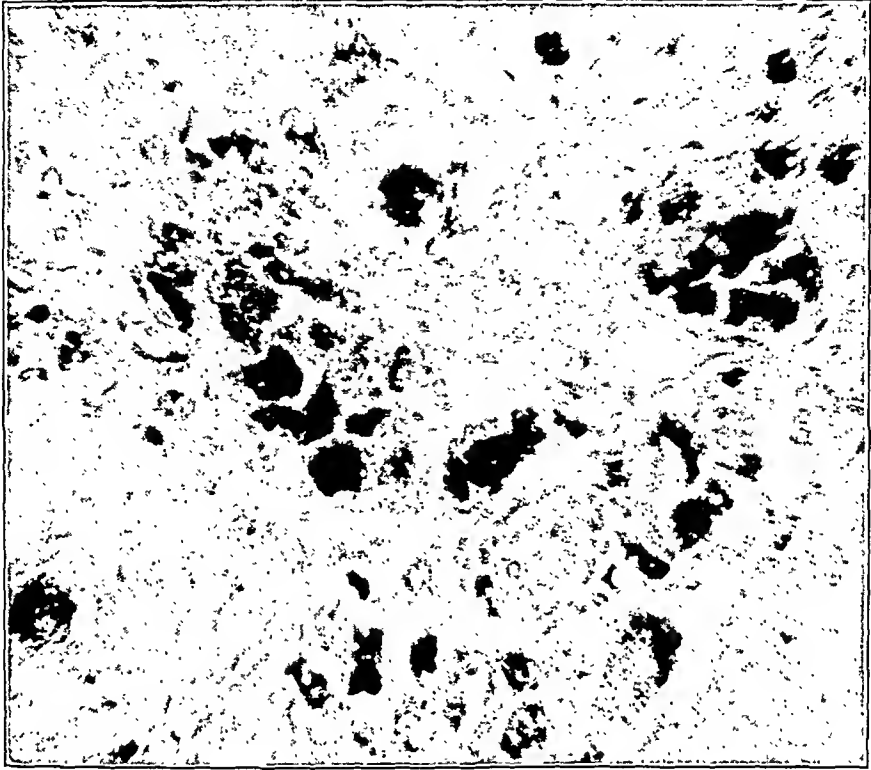
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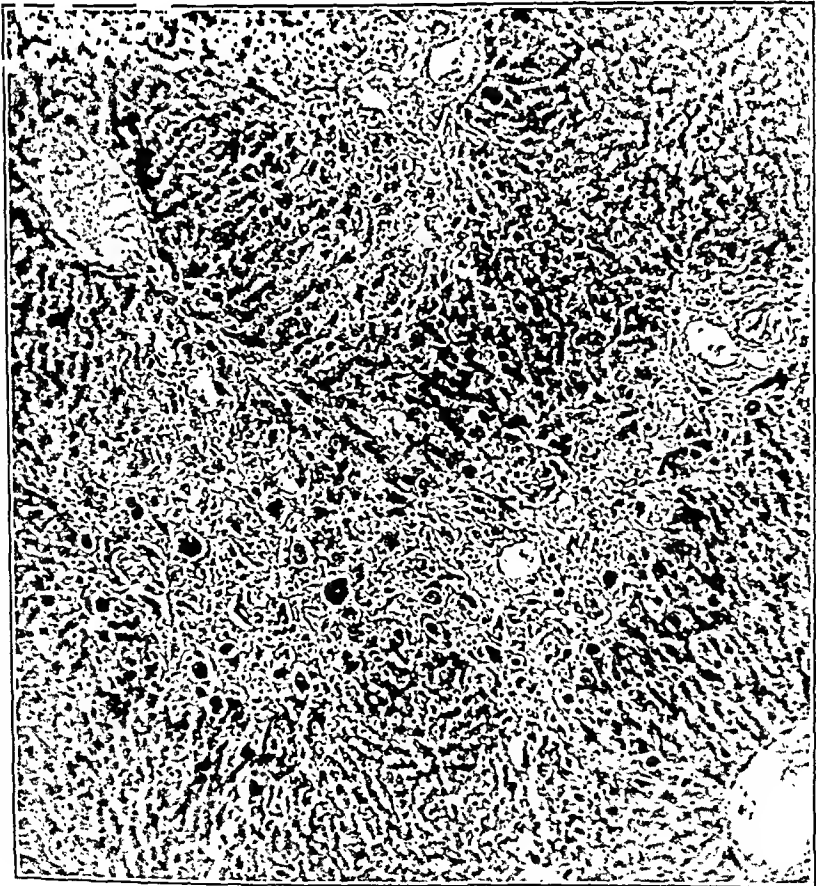
PLATE 62

FIG. 5. Rabbit 38, fed copper acetate for 63 days. Photomicrograph of liver under oil showing large Kupffer cells loaded with hemofuscin. Hematoxylin, ammonium sulphid and fuchsin stain.

FIG. 6. Rabbit 38, showing a low power photomicrograph of the liver with the development of a fine intralobular cirrhosis. Hematoxylin and eosin stain.

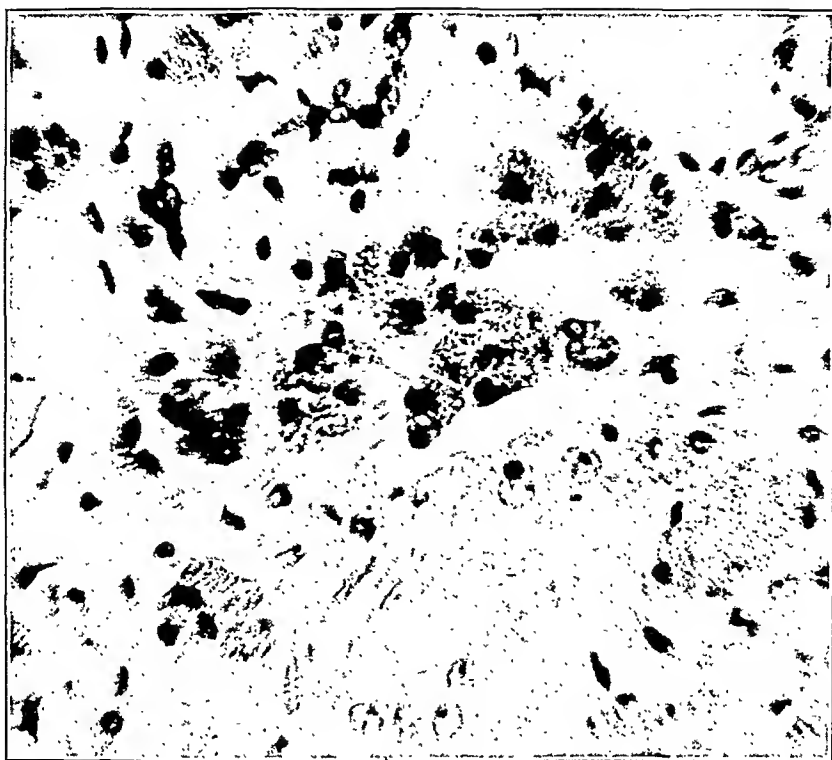


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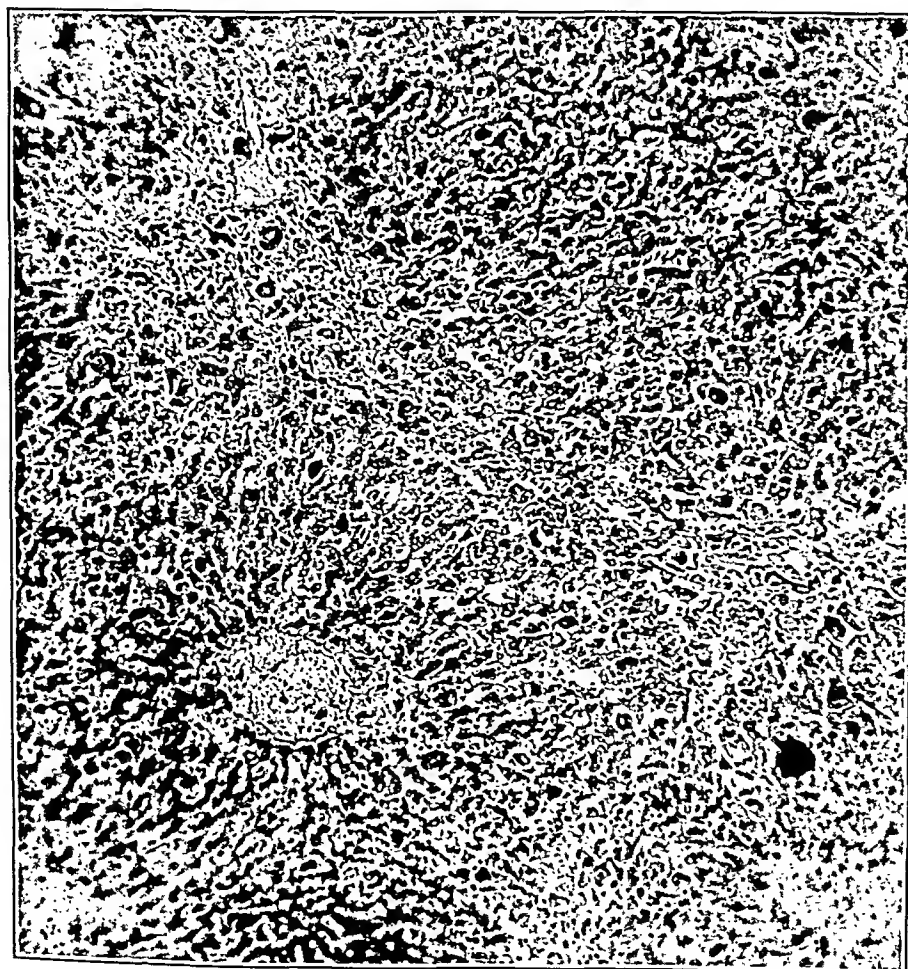


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Experiments were carried out in which a number of white rats were fed on the yellow beef livers. The rats developed parenchymatous degeneration and necrosis of the liver cells with more or less round cell infiltration. Since, according to Wells and Hedenburg,<sup>4</sup> Connor<sup>5</sup> and others, carotin is considered non-toxic, Buckley and his associates thought that some unknown toxic agent was the real offender, while carotin had accumulated in the liver due to damage to the liver cells.

The investigations of Buckley, *et al.*, are presented here at some length because the photomicrographs which they publish are so strikingly like the histological pictures seen in some of our rabbit livers following a diet heavy in carrots.

It has seemed desirable in view of the claims of Flinn and Von Glahn and of Polson to investigate the effect of carrot-feeding on the livers of rabbits. The necessity of adequate controls in experiments of this kind has been emphasized in the preceding paper.<sup>6</sup>

## METHODS

As before, litter-mates of the same sex have been compared, the only difference in the treatment of the two animals of each pair being a high percentage of carrots in the diet of one series. The various diets which were used are detailed in Table I.

Alfalfa bloom is prepared by grinding the dried leaves of the alfalfa plant, the stems and stalks being discarded. The fresh carrots used were the ordinary field variety which are rich in carotin. They were washed with a stiff brush until free of dirt and most of the skin. In the first experiment summer carrots were used and in the second winter carrots. There was no obvious difference between them. The dried carrot was a high-grade product prepared by drying sliced carrots in a current of recirculated air at a relatively low temperature.

The rabbits were killed in four different lots after the experiment had been in progress 32, 42, 47, and 50 days respectively. The various organs were examined grossly and blocks of liver tissue were fixed in 95 per cent alcohol. The blocks were embedded in paraffin and the sections stained with a weak solution of basic fuchsin following treatment with potassium ferrocyanide and hydrochloric acid. Sections were also stained with hematoxylin and eosin and with eosin-methylene blue. The pigment granules in the Kupffer giant cells were stained more satisfactorily with the methylene blue than with the basic fuchsin.

## EXPERIMENTAL HEPATIC PIGMENTATION AND CIRRHOSIS \*

### II. THE EFFECT OF HEAVY CARROT-FEEDING ON THE RABBIT'S LIVER

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Flinn and VonGlahn<sup>1</sup> have recently claimed that a diet consisting exclusively of carrots will produce marked pigmentation of the liver in rabbits. In fact, they state that pigment production is more readily produced by the feeding of carrots than by the feeding of copper. Although they present no data to prove the nature of this pigment, they say that it is in every way identical with that found in copper poisoning. Polson,<sup>2</sup> working in Great Britain, reports having obtained pigmentation of the livers of rabbits in 88 per cent of his animals by feeding carrots, oats and thirds. He describes the pigment as hemofuscin.

Buckley, Joss, Creech and Couch<sup>3</sup> have recently (1930) reported a condition in cattle which they designate "carotenosis of bovine livers." This condition was discovered in the course of meat inspection at five different slaughtering centers ranging from Buffalo, N. Y., to Phoenix, Arizona. The livers of these animals were found to be an intense yellow or reddish yellow color. When the fresh livers are sectioned the knife and fingers are stained a deep yellow. As the condition advances fibrous changes develop in the liver, in the more severe cases culminating in advanced cirrhosis. No pigment deposit in the form of granules or larger solid particles is described. The condition appears to be confined to the liver, as all other organs were normal in their animals. Chemical studies of the livers yielded a substance which was identified as "carotene" by chemical and boiling point determinations and spectrophotometric measurements. Carotin in amounts up to forty times that found in the normal bovine liver was obtained.

\* Received for publication June 2, 1931.

TABLE II

*Summary of Results in Carrot-Feeding Experiment No. I, using Control Diet No. I and Carrot Diet No. I*

No.	Diet group	Sex	Variety	Litter No.	Days on diet	Gross body weight	Net body weight	Liver weight	Liver per kg.	Pigments		Cirrhosis
										Hemo-fuscin	Hemo-siderin	
102	Control 1	F	Albino	1	47	Kg. 2.12	Kg. 1.58	gm. 80	gm. 50	..	..	..
107	Control 1	M	Albino	2	47	2.27	1.47	96	65	+	-	-
108	Control 1	M	Albino	3	47	1.28	0.85	51	60	±	++	-
109	Control 1	M	Albino	3	47	1.68	1.24	71	57	..	..	..
103	Carrot 1	F	Albino	1	47	1.52	1.14	70	61	-	-	-
105	Carrot 1	M	Albino	2	47	1.06	0.52	85	163	+	-	-
112	Carrot 1	M	Albino	3	47	1.80	1.30	95	79	-	-	-
113	Carrot 1	M	Albino	3	47	1.96	1.28	101	80	+	±	-

### GROSS CHANGES IN THE LIVER

The only gross changes evident in the livers of the carrot-fed group were the increase in the size of the livers in the group fed on fresh carrots and the yellow to yellowish red color of the livers. There was no evidence grossly of fibrous tissue changes.

### HISTOLOGICAL CHANGES IN THE LIVER

In the first experiment (Table II) practically no pigmentary changes were found. One of the controls, Rabbit 108, showed a moderate number of hemosiderin granules in the liver cells, as shown by the Berlin blue reaction. One of the carrot-fed group, Rabbit 113, showed a trace of hemosiderin. Two of the controls and two of the carrot-fed group showed a few granules of hemofuscin in the liver cells, no more than occurs physiologically in many rabbit livers. No fibrous tissue proliferation was found except that produced about the bile ducts in the coccidia-infested animals. It is evident that these results are quite different from those produced under similar conditions by the feeding of copper acetate.<sup>6</sup>

Four apparently normal albino rabbits were fed from the time they were 60 until they were 107 days of age, a period of 47 days, on Control Diet No. 1. Four litter-mates of the same sex received Carrot Diet No. 1 over precisely the same period. The results of this experiment are presented in Table II.

TABLE I

*Composition of the Various Diets Employed*

Experiment	Diet	Per cent
Control Diet No. 1 .....	Alfalfa bloom	25
	Water	75
Carrot Diet No. 1 .....	Alfalfa bloom	20
	Ground fresh carrot	80
Control Diet No. 2 .....	Alfalfa bloom	75
	Ground barley	25
Carrot Diet No. 2 .....	Fresh carrot (plus barley)	100
Dried Carrot Diet .....	Dried ground carrot	90
	Alfalfa bloom	5
	Ground barley	5

Nine rabbits 60 days of age were started on each of the last three diets described in Table I. For each rabbit on Control Diet No. 2 there was a litter-mate of the same sex upon Carrot Diet No. 2 and another on the Dried Carrot Diet. A third of the rabbits in each group were killed in 32 days, a third in 42 days and the remainder after 50 days on the diets. The rabbits on the fresh carrot diets received only washed or peeled fresh carrots with the addition on the 1st, 6th, 12th, 22nd and 35th days of feeding of 100 grams of oats for each three rabbits.

An interesting but as yet unexplained observation is the increase in the size of the liver of the rabbits on the fresh carrot diets. This is well shown in the averages comprising Table IV and is the most evident in those rabbits which received fresh carrots for the longest periods. In the first experiment (Table II) the mean quantity of liver per kilogram of net body weight is 58 grams while the average of those receiving chiefly fresh carrot is 96 grams.



In Experiment 2 (Table III) one control and five carrot-fed rabbits show small numbers of iron-free granules in the liver or in large Kupffer cells. Rabbits 124, 128 and 116 show a number of Kupffer giant cells composed of several agglomerated cells. These cells contain a moderate number of yellowish brown granules which do not stain well with basic fuchsin but stain quite readily with methylene blue (Fig. 3). The production of Kupffer giant cells is quite similar to the early lesion produced in the rabbit's liver by the ingestion of copper salts.<sup>6</sup>

TABLE IV

*Showing the Mean Relative Increase in Liver Weight of the Rabbits Fed on Fresh Carrots as Compared with the Controls and Those Fed on Dried Carrots*  
(Grams Liver per Kg. of Net Body Weight)

Days on diets	Control Diet No. 2	Fresh Carrot Diet No. 2	Dried Carrot Diet
32	49	44	56
42	47	62	59
50	49	70	59

The number and size of the Kupffer giant cells in the carrot-fed animals are greatly reduced as compared with similar giant cells produced by the copper-fed animals in the same periods of time. The giant cells in the carrot-fed group are scarcely discernible under the low powers of the microscope, while in the case of the copper-fed animals, immense cells loaded with pigment can be seen in great numbers under this magnification. An idea of the relative amounts of pigment in the livers of the carrot-fed and copper-fed rabbits may be obtained by comparing Fig. 3 with Figs. 1, 3 and 5 of our previous paper.<sup>6</sup> Of the twenty-two rabbits fed on the various carrot diets, only three developed giant cells containing pigment, while seventeen out of twenty-one copper-fed rabbits developed Kupffer giant cells heavily loaded with hemofuscin.

A moderate amount of hemosiderin was also seen in the liver of Rabbit 128. Blue granules were found in the hepatic cells as well as in the giant Kupffer cells. This animal likewise presented a fairly definite cirrhosis and fatty infiltration of the liver cells (Fig. 1). This is the only animal in the entire group of 22 that received carrots which shows a definite early cirrhosis. In Rabbits 124 and 130 a beginning fibrous proliferation can be discerned.

TABLE III

Summary of Results in Carrot-Feeding Experiment No. 2, using Control Diet No. 2, Fresh Carrot Diet No. 2, and Dried Carrot Diet

No.	Diet group	Sex	Variety	Litter no.	Days on diet	Gross body weight Kg.	Net body weight Kg.	Liver weight gm.	Liver per kg. gm.	Pigments		Histology	
										Hemo-fuscin	Hemo-siderin	Cirrhosis	Other changes
114	Control 2	M	Albino	1	32	1.70	0.45	59	47	-	-	-	None *
123	"	M	"	2	32	1.62	0.50	55	50	-	-	-	None
126	"	M	"	3	32	1.40	0.40	52	52	-	-	-	None
117	"	F	"	1	42	1.55	0.40	57	50	-	-	-	Coarsely granular cytoplasm
120	"	F	"	2	42	1.90	0.45	65	45	-	-	-	None
127	"	F	"	3	42	1.60	0.40	55	46	-	-	-	Vacuolate cytoplasm
132	"	F	"	4	50	1.90	0.50	67	50	-	-	-	Cytoplasm granular
134	"	F	Brown	5	50	1.45	0.38	54	50	-	-	-	Coarsely granular cytoplasm
136	"	M	Black	6	50	1.55	0.40	54	47	+	-	-	Coarsely granular cytoplasm
115	Fresh Carrot 2	M	Albino	1	32	0.98	0.35	21	33	+	+	-	Granular cytoplasm, liver cells swollen
124	"	M	"	2	32	1.15	0.37	45	57	+	-	+	Few Kupffer giant cells, fatty infiltration
128	"	M	"	3	32	1.44	0.43	41	41	+	++	++	Large Kupffer giant cells, fatty infiltration
118	"	F	"	1	42	0.95	0.38	36	62	-	-	-	None
121	"	F	"	2	42	1.40	0.40	69	69	-	-	-	Vacuolate cytoplasm
129	"	F	"	3	42	1.40	0.39	56	55	-	-	-	Vacuolate cytoplasm
133	"	F	"	4	50	1.70	0.45	103	81	-	-	-	Vacuolate cytoplasm, early nuclear changes
137	"	F	Brown	5	50	1.55	0.40	82	75	-	-	-	Vacuolate cytoplasm, early nuclear changes
139	"	M	Black	6	50	1.28	0.33	52	55	-	-	-	Vacuolate cytoplasm
116	Dried Carrot	M	Albino	1	32	1.32	0.45	52	50	+	-	-	Kupffer cells slightly enlarged
125	"	M	"	2	32	1.40	0.45	46	48	-	-	-	None
130	"	M	"	3	32	1.77	0.55	75	62	-	-	+	None
119	"	F	"	1	42	1.00	0.35	36	55	+	-	-	Kupffer cells slightly enlarged, occasional mitoses
122	"	F	"	2	42	1.30	0.45	50	60	-	-	-	Coarsely granular cytoplasm
131	"	F	"	3	42	1.40	0.40	62	62	-	+	-	Granular, vacuolate cytoplasm
135	"	F	"	4	50	1.55	0.45	74	67	-	-	-	Coarsely granular cytoplasm
138	"	F	Brown	5	50	1.42	0.36	58	54	-	-	-	Vacuolate cytoplasm, nuclei pale staining
140	"	M	Black	6	50	1.50	0.38	62	56	-	-	-	Coarsely granular cytoplasm

\* Practically all of the livers show more or less coccidiosis.

## SUMMARY

1. Twenty-two rabbits, divided into three groups, were fed on three different diets, all of them containing a high percentage of carrots.
2. Litter-mate controls were used throughout.
3. The duration of the experiments was from 32 to 50 days.
4. Three animals developed Kupffer giant cells which contained a moderate amount of hemofuscin.
5. One rabbit developed a definite early cirrhosis; two other animals showed slight connective tissue proliferation in their livers.
6. Many of the animals showed clear, vacuolated cytoplasm in the liver cells probably due to excess glycogen storage.
7. Other changes include parenchymatous degeneration, fatty infiltration and early nuclear degenerative changes. These conditions may be dependent upon diet-deficiency due to the almost exclusive diet of carrots.

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6. Hall, E. M., and MacKay, E. M. Experimental hepatic pigmentation and cirrhosis. I. Does copper poisoning produce pigmentation and cirrhosis of the liver? *Am. J. Path.*, 1931, 7, 327.

## DESCRIPTION OF PLATE

## PLATE 63

- FIG. 1. Rabbit 128. Photomicrograph of liver showing early cirrhosis with fatty infiltration. The Kupffer giant cells are scarcely discernible. Hematoxylin and eosin. Low power.
- FIG. 2. Rabbit 133. Photomicrograph showing swollen liver cells with clear cytoplasm and beginning nuclear changes. Hematoxylin and eosin. High power.
- FIG. 3. Rabbit 128. Photomicrograph showing Kupffer giant cells containing 8 to 10 nuclei and a few granules of hemofuscin in the cytoplasm. Eosin and methylene blue. Oil immersion.

Animals fed on fresh carrots showed greater changes in the liver than did those fed on the dried substance. This may have been due to the greater consumption of the fresh vegetable.

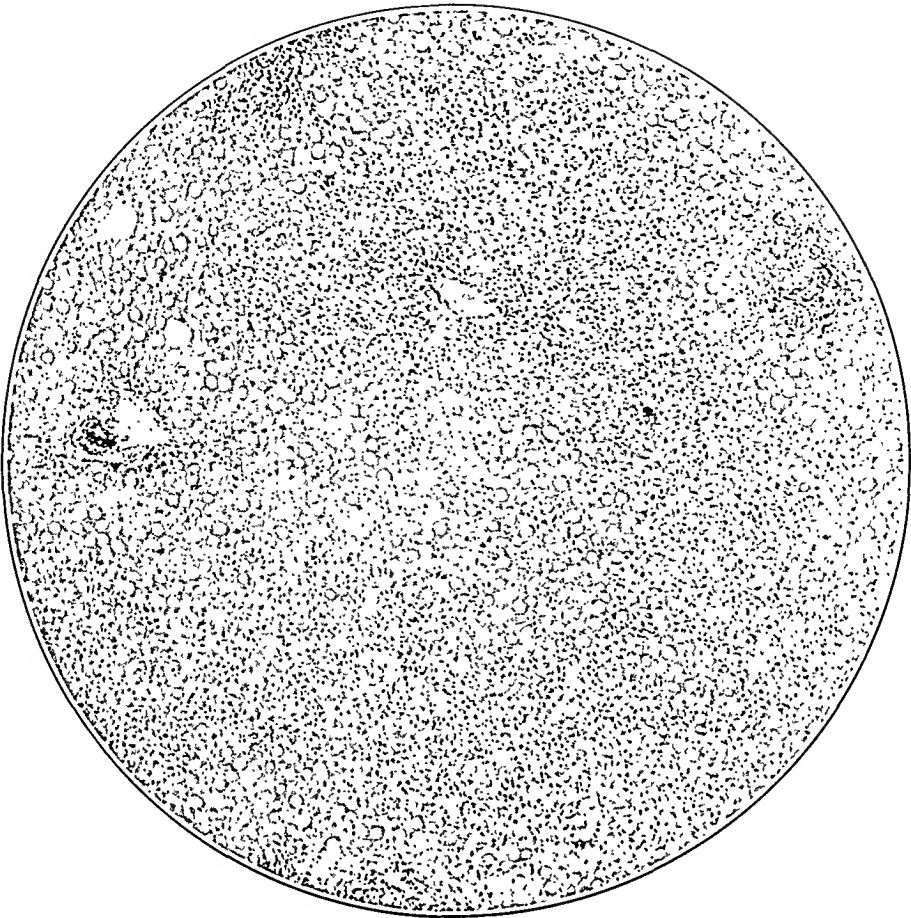
### COMMENT

Besides the pigmentary and fibrous changes described, many of the livers presented more or less disturbance of the cytoplasm of the hepatic cells. In the greater number of animals the cytoplasm appears to be more coarsely granular than is usually seen. Since four of the control animals show a similar condition, no great emphasis can be placed on this finding. In a number of animals, *viz.*, 118, 121, 129, 133, 137, 131 and 138, the cytoplasm is vacuolated, resembling the effects of a fatty or hydropic degeneration (Fig. 2). One of the controls, Rabbit 127, shows a similar change. It will be noted (Table III) that four of the fresh-carrot group, two of the dried-carrot and one control exhibit vacuolation of the cytoplasm. Such a condition might easily be the result of excess glycogen\* stored in the liver cells. The livers of well nourished normal rabbits quite generally show a similar although less advanced condition.

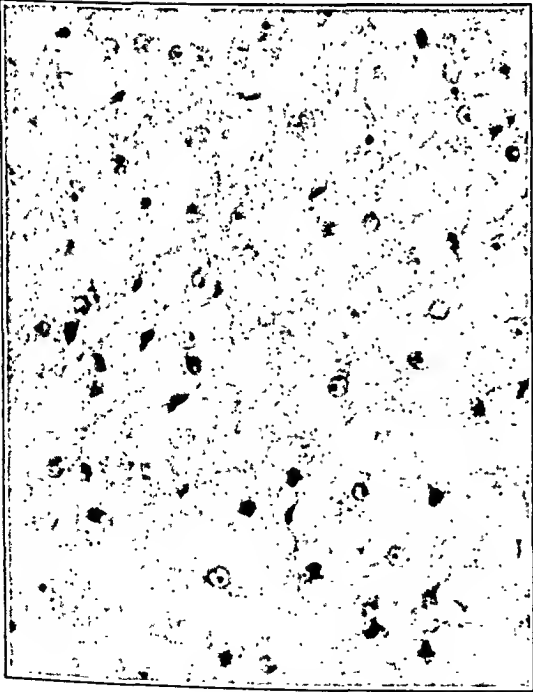
None of the carrot diets (Table I) are balanced diets and are, no doubt, deficient in certain food essentials, especially protein. The cytoplasmic changes and possibly also the fibrous tissue proliferation may be dependent upon this deficiency. Since carotin is considered by most investigators to be non-toxic it seems logical to assume that lack of certain vitamin or other food essentials may well be the active factor concerned. Whether or not this be the true explanation, it is evident that this type of feeding experiment is difficult if not impossible of interpretation. Obviously if the diet is deficient in any essential food substance no one can say whether results produced are due to the action of carotin or due to the lack of some food essential. Further investigation of the nature of the above changes is now under way.

\* Best's carmine stains of the liver in one of the animals fed dried carrots shows all of the liver cells loaded with glycogen. The glycogen granules are large, vary considerably in size and quite evidently occupy the spaces in the cytoplasm which are seen in ordinary sections. The hematoxylin and eosin stain shows clear vacuolated cells similar to those shown in Fig. 2. The control liver shows considerable glycogen in the central two-thirds of the lobule with the cells of the periphery free. The granules are uniform in size and evenly distributed. One of the fresh carrot livers reveals glycogen in amounts between the control and the one fed dried carrots. Ordinary stains of the fresh carrot and control animals fail to show the vacuolate appearance of the cytoplasm.

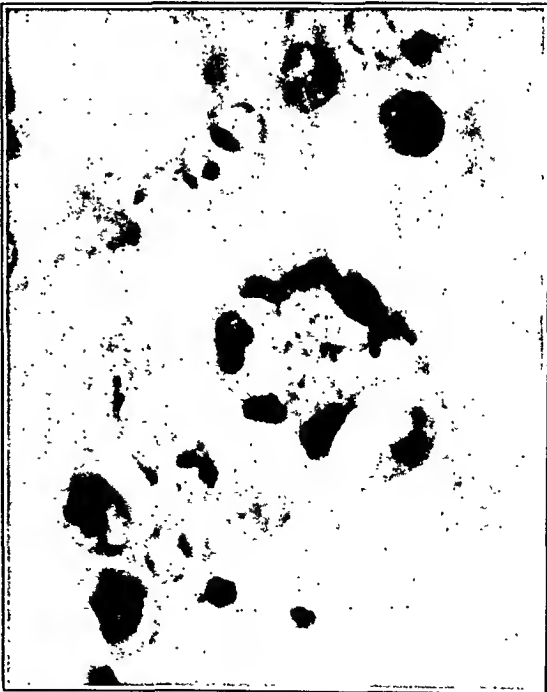




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## EXPERIMENTAL WORK

For the past two years we have been studying the lesions produced by acute and chronic poisoning with copper, paying attention especially to the origin and nature of the pigmentation which always occurs in the liver. The animals used were rabbits, sheep, monkeys and guinea pigs.

The copper was administered in the form of metallic powder (electrolytic, Eimer & Amend) suspended in lard warmed before use to body temperature, and was injected subcutaneously by means of a syringe and needle. The lard and body juices readily dissolve the powder at a sufficiently rapid rate for the needs of the experiment. The method is much simpler, surer and more accurate than putting powder on food or using any of the copper salts and introducing them through a stomach tube or mixed with the food. As the suspension ages it acquires a bluish green color due to solution of copper and is on this account more toxic than a suspension freshly made. This point should be borne in mind.

A 20 per cent suspension of the metallic powder in lard was ordinarily used. 1 cc. therefore equalled 200 mg. of copper. The dose varied from this up to 1 gm. and was repeated every four weeks if the animal survived. It was found advisable to inject the dose in small amounts at several different sites so as to avoid the formation of a sterile abscess and sloughing. Young rabbits were used and averaged about 1500 gm. in weight at the beginning of the injections. A dose of 1 gm. usually killed a rabbit with acute lesions in two to four weeks, but one rabbit which received one dose of 2 gm. survived eight weeks.

It cannot be too strongly emphasized that a sufficient dose of copper must be administered in order to reproduce our results. If a certain minimum is not exceeded, the animal can handle it with no injurious effects. As examples of the importance of dosage, we cite the records of two animals in our series. The first is a rabbit which had been receiving copper in the form of a solution of the acetate on its food daily for three years and two months. At that time a piece of liver was removed for microscopic study and was found to show a considerable number of coarse pigment granules at the peripheries of the lobules, but no cirrhosis or necrosis. The animal was then given powdered metallic copper sprinkled in generous

## EXPERIMENTAL COPPER POISONING \*

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### INTRODUCTION

In 1921 Mallory, Parker and Nye<sup>1</sup> announced that it was possible to produce pigmentation and cirrhosis of the liver in rabbits and sheep by administering to them copper salts or metallic copper in powdered form. Their results have been confirmed by Hall and Butt<sup>2</sup> but denied by Flinn and VonGlahn<sup>3</sup> and by Polson,<sup>4</sup> who claim that copper does not produce either pigmentation or cirrhosis and that the pigmentation present is due to a diet of carrots or similar vegetable.

Oshima and Siebert<sup>5</sup> and more recently Herkel<sup>6</sup> have attempted in vain to duplicate our results and Herkel was compelled to reach the conclusion that it is not possible to produce cirrhosis of the liver in rabbits and rats by the use of copper. The pigmentation in the rabbit's liver, he adds, occurs physiologically. At the same time he acknowledges that this natural pigment is not identical with that in the livers of our rabbits, sections of which he has studied, and states that we have without doubt produced in certain animals severe changes in the liver in the sense of degeneration followed by connective tissue proliferation.

What is the cause of these two diametrically opposed views; one, that copper produces pigmentation and cirrhosis of the liver in rabbits; the other, that it produces neither? It may depend on one of two different factors: first, that the dose of copper which actually gets into the tissues is too small, the other, that there are breeds of rabbits in which, as in guinea pigs, the blood and liver are apparently immune to the action of copper.

Proof that the natural pigment occurring in the livers of rabbits and that due to the toxic action of copper are entirely different chemically would necessarily settle immediately one of the points in dispute. One of the objects of this paper is to present such proof.

\* Presented April 3, 1931, before the American Association of Pathologists and Bacteriologists at Cleveland, Ohio.

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The leucocyte count tends to be low, averaging 3000 to 6000 cells per cmm. There is often a decrease in the number of polymorphonuclear cells accompanied by a rise in the monocytes. An occasional metamyelocyte can sometimes be found.

In contrast to rabbits, guinea pigs develop no anemia, even if the dose of copper be such that the animal dies in a few days. There seems to be a tendency to a rise in the reticulocytes and, in some instances, an increase instead of a decrease in the hemoglobin and erythrocytes.

The blood of two monkeys was examined three days after the injection of copper. One animal showed a drop of 600,000 red cells and the other a decrease of 1,800,000 red cells per cmm. below the counts taken before injection. In both animals, the reticulocytes were more than doubled.

The bone marrow of rabbits dying at various times after injection with the metal never shows any degeneration or necrosis. In the acute stages, there is usually marked activity of both the erythroblastic, granulocytic and megakaryocytic series.

The spleens on the whole from our series of animals have shown no definite pathological changes. However, the spleen from a rabbit killed forty-eight hours after injection and with an anemia of 2,080,000 red cells per cmm. showed many clumps of non-hemoglobin-containing red cells. Most of these clumps occurred free in the pulp but a certain number had been phagocyted by macrophages.

*Kidney:* A certain percentage of our animals had hemoglobinuria at the time of death. The urine from such animals was dark brown in color. Spectroscopic examination revealed a large amount of oxy-hemoglobin and a trace of methemoglobin. Histologically the kidneys showed numerous casts in the tubules. These casts varied in color from greenish to red in sections stained with eosin-methylene blue. In structure they were solid, hyaline casts or were hollow cylinders or a series of globules approximately the size of red blood corpuscles. We do not know the composition of the material composing these casts although in the past it has been assumed to be hemoglobin. This material was not found in the capsular spaces of the glomeruli. Copper could not be demonstrated in such casts. Associated with the occurrence of these casts, and the concomitant hemoglobinuria, was necrosis of the tubular epithelium.

amounts on its food each day. Nine months later it died and showed extensive pigmentation and cirrhosis and tubular nephritis. The second animal, a monkey, was injected at monthly intervals with 2 cc. of a 4 per cent suspension of copper over a period of seven months. A piece of liver was then removed and was completely negative microscopically. The dose administered was increased to 5 cc. of a 20 per cent suspension of copper. The animal died nine months later and its liver then showed extensive pigmentation, the pigment occurring in liver cells and macrophages.

Sheep are so susceptible to copper that a 4 per cent suspension had to be used for them in order to make the dose small enough (1 cc. equals 40 mg.).

### *Acute Poisoning*

If copper is given in a sufficiently large dose, it will kill a rabbit in from twenty-four hours to two or three weeks. We have considered any animal dying within three weeks after injection as dying of acute poisoning. In our description of the pathological changes, we shall consider the blood, bone marrow, spleen, kidney and liver in the order named, reserving a separate section for discussion of pigmentation.

*Blood, Bone Marrow and Spleen:* In rabbits, twenty-four to forty-eight hours after the injection of copper, there occurs a fall in the red blood cell count accompanied by a corresponding fall in the hemoglobin and a rise in the reticulocytes. The lowest level of the erythrocyte count is usually reached four days after the injection, the number of red cells varying from two and a half to three million per cmm. with a similar reduction in the hemoglobin. At this time, the reticulocytes show a marked rise, often reaching 20 or 30 per cent, in one instance 41 per cent, and it is not unusual to find an occasional nucleated red blood cell. Following this period, the erythrocyte count and hemoglobin tend to return gradually to a normal level, accompanied by a decrease in the number of reticulocytes. This normal level is reached in two to four weeks. Smears of the blood show no stippling or other abnormalities of the red cells except for the rather large basophilic cells, the reticulocytes.

Spectroscopic examination of the serum or plasma at the height of the anemia has consistently failed to reveal any significant hemoglobinemia; the trace of hemoglobin present is probably to be accounted for by trauma incident to taking the sample of blood.

When the process is less marked, single liver cells or small groups of them scattered throughout the lobules are affected. The necrotic cells are invaded and removed by macrophages. The lesion is apparently confined to the liver cells, not affecting the bile duct epithelium or the tissues composing the stroma.

*Pigmentation:* The livers of normal rabbits frequently contain varying amounts of pigment in the form of yellow granules. An apparently similar pigment occurs also in large amounts in the mesenteric lymph nodes. This natural liver pigment is increased with age and also as a result of acute and chronic infections. It stains with basic dyes such as fuchsin but not with fat stains such as Sudan IV or oil red O, differing in this respect from the so-called waste pigment found in humans.

Up to the present time there has been no method of distinguishing between this natural pigment and the pigment occurring after copper poisoning and this fact has undoubtedly led to confusion and scepticism on the part of other workers who have tried to repeat our work. However, recently we have found that the application of a method which has long been known clears up this difficulty completely. The method depends on the fact that a freshly prepared solution of hematoxylin stains copper a deep blue to blue-black. The details of this are given in the section on technic.

Application of this stain revealed the fact that the pigment granules which appear in the liver cells twenty-four hours after the injection of copper contain copper. At two weeks there are numerous pigment granules which stain positively for copper. From this time on, up to two months after injection, copper could be demonstrated in the pigment granules. However, at this point the staining of the granules had become less intense, indicating that the metal was disappearing. At five months, the copper had completely disappeared from the granules and the pigment either did not stain at all or stained a brownish black, indicating the presence of iron. This was confirmed by the ferrocyanide reaction for iron. The natural pigment of the rabbit liver is not stained at all by hematoxylin.

Therefore, by means of this method, we have been able to show that copper is present in the pigment granules following the injection of copper, and that such pigment granules, after the copper leaves them, begin to show unmasked iron. These two findings would seem to answer the contention of some other workers that the pigment

In a certain number of animals that showed no hemoglobinuria at the time of death, similar casts could be found in groups of tubules in the cortex, undoubtedly representing a late or healed stage of hemoglobinuria. Experiments on phenylhydrazine poisoning support this explanation.

In kidneys of rabbits showing neither hemoglobinuria nor necrosis of the tubular epithelium, evidence of injury to the epithelium of the convoluted tubules was present in the form of hyaline or colloid droplets in the cytoplasm of the cells.

In the guinea pigs in our series, hemoglobinuria occurred in only one animal and that was one that died forty-eight hours after injection. Necrosis of the tubular epithelium in guinea pigs dying of acute poisoning was not uncommon.

In the other species of animals employed in our experiments, that is, monkeys, sheep and rabbits, hemoglobinuria was quite common and especially so in the sheep.

*Liver:* The liver in acute poisoning shows two lesions of importance — pigmentation and necrosis. As early as twenty-four hours after injection, a considerable number of pigment granules can be found in the liver cells, especially at the peripheries of the lobules. These granules contain copper and form the characteristic pigment of copper poisoning, as will be discussed later. From twenty-four hours on, these pigment granules continue to increase in number until at two weeks the pigmentation is marked and can be recognized grossly as well as microscopically. Grossly such a liver is a golden brown. Microscopically the pigment granules are found in the liver cells, and, as a rule, in macrophages throughout the lobules and in the portal areas. The majority of the pigment-containing liver cells appear normal, but a certain number are degenerating and others are definitely necrotic. Many of the necrotic cells do not contain pigment, indicating that copper, and not the pigment itself, is the cause of the necrosis. The pigment in the macrophages represents that taken up during the removal of dead, pigmented cells.

Necrosis of the liver cells appears later than the pigmentation. The time of its first appearance has not been definitely determined, but two weeks after injection it is present and conspicuous. Its degree and location vary somewhat in different animals. When the injury is severe, all the liver cells at the centers of the lobules are involved, giving rise to the picture of an extensive central necrosis.

will not stain copper well, apparently because the metal is dissolved out of its compounds.

3. Wash sections in tap water, dehydrate in alcohol, clear in organum oil or xylol and mount in xylol balsam.

Copper compounds are stained blue to blue-black, hemosiderin brownish black to black. The stain seems to be permanent.

*Note.* The staining solution is made by adding a little hematoxylin (as much of the crystals or powder as will cover the point of a small scalpel) to 10 cc. of water, which must be neutral, and dissolving with the aid of heat. A strength of about 0.5 per cent is desirable. Distilled water is almost always acid and gives with hematoxylin a yellow solution.

The best and most reliable hematoxylin solution is made by using a mixture of solutions of monopotassium and disodium phosphates with a pH of 7.0. The color of the hematoxylin dissolved in this buffered solution is a rich red owing to the presence of the buffer salts.

A counterstain in a 0.25 per cent solution of basic fuchsin in 50 per cent alcohol is sometimes advantageous as it brings out the granules which contain no copper or stainable iron. Stain the sections in the fuchsin solution for 5 to 20 minutes, differentiate well in alcohol, and clear and mount as before.

### *Triple Nitrite Test for Copper*

1. Blot a frozen, celloidin or paraffin section of alcohol-fixed liver tissue on a slide.
2. Place on it a crystal of sodium acetate.
3. Add one or two drops of a saturated solution of potassium nitrite.
4. Add one or two drops of a 1 per cent solution of acetic acid.
5. Dissolve a minute amount of lead acetate in the above mixture.
6. Cover the section with a cover glass and watch under the microscope for the development of square dark yellow crystals on the surface of the tissue. As they enlarge they appear black.

### DISCUSSION

We feel that the results of acute poisoning with copper described above both throw light on the mechanism of copper poisoning and also support our former claims as to the effect of copper in producing

observed by us was natural pigment or pigment due to certain substances in the diet.

Moreover, a corroborative microchemical method has been employed by us, namely, the triple nitrite test for copper, the technic of which is given below. This test was applied to sections of the same livers on which we used the hematoxylin stain. By means of this microchemical reaction, we have been able to demonstrate the presence of copper in sections of livers from acutely poisoned rabbits, on which the hematoxylin stain was likewise positive for copper. Thus, by means of the triple nitrite test, we showed that copper was present in such livers and, by staining with hematoxylin, that it was located in the pigment granules, and, to a less extent, in the cytoplasm of the hepatic cells. The triple nitrite test is negative with normal livers, not being sufficiently sensitive to detect the amount of copper normally present.

### *Chronic Poisoning*

The pathology of this form of poisoning has been described in detail in our former report. In brief, the two outstanding lesions are pigmentation and cirrhosis. Our latest findings in regard to the method of production of the pigment and its characteristics have been described in the section on pigmentation. The cirrhosis is the result of repeated necrosis of the liver cells following each injection of copper and of an increase in stroma. This increase in stroma occurs partly as a result of condensation where necrotic liver cells have not been replaced and partly due to new formation of connective tissue and blood vessels to serve as stroma for islands of newly formed liver cells. Grossly, such a liver is firm and its surface is finely granular. Some of our animals which had cirrhosis showed jaundice, and in one instance ascites.

### TECHNIC

#### *Hematoxylin Stain for Copper*

1. Fix tissue, cut in thin slices, in 95 per cent alcohol or in 10 per cent formalin, buffered to a pH of 7.0.
2. Stain frozen, celloidin or paraffin sections in a freshly prepared, neutral aqueous solution of hematoxylin for one hour or longer. A solution which is acid or more than very faintly alkaline

is the occurrence of hemoglobinuria, the anemia and the fact that the spleen of a rabbit forty-eight hours after injection contained masses of hemolyzed red cells. The hemoglobin thus liberated is in part excreted by the kidneys and in part taken up by the liver. That we have not been able to demonstrate a definite hemoglobinemia probably can be explained by the supposition that the hemoglobin, as fast as it is set free, is disposed of in these two ways. Since no hemoglobin can be found in the glomerular capsular spaces of kidneys containing hemoglobin casts, it must be excreted in a dilute form and concentrated in the tubules.

The copper, as fast as it penetrates the body fluids, is stored in the liver, and we have found it there twenty-four hours after injection of the metal. Herkel has shown that by far the greater part of the copper in experimental poisoning is stored in this organ. Since no copper can be demonstrated in the hemoglobin casts, it is probably absorbed as such and not as a combination with hemoglobin. Thus the metal and some form of hemoglobin or a derivative are taken up separately by the liver. In the liver cells a compound of the two substances forms, giving rise to the pigment granules characteristic of this form of poisoning.

In addition to taking part in the formation of this pigment, copper also has a toxic action on the liver cells, leading to degeneration and necrosis. It has a similar effect on the tubular epithelium in the kidney. In this organ the injurious action is not dependent on the often associated hemoglobinuria, for tubular nephritis is common in the guinea pig, an animal in which hemoglobinuria is very rare, and also tubular degeneration is an almost constant finding in the kidneys of rabbits in which no hemoglobinuria or only a healed stage of the process is present.

Copper, like lead, acts on the circulating red cells and not on the marrow. Histological examination of the marrow has shown no evidence of degeneration or necrosis, but, on the other hand, an active hyperplasia.

In the liver the copper persists for several weeks following administration, but at the end of two months it is definitely decreased in amount, a certain percentage of granules in such a liver containing no copper while others contain a much lessened amount. The copper presumably is excreted through the bile, a point that will be considered in our second paper. At the end of five months copper is no

pigmentation and cirrhosis of the liver. We will take up first a discussion of the mode of action of copper in experimental poisoning and then review our present results in relation to our former findings.

One of the most important effects of acute copper poisoning from our point of view is the changes produced in the blood. Since we felt that the pigment formed as a result of poisoning with copper represented some form or derivation of hemoglobin, it was essential that we demonstrate that copper has an effect on the hemoglobin in the animal body. That copper hemolyzes red cells *in vitro* has long been known, but in our previous work on chronic copper poisoning, none of the animals showed anemia or other evidence of blood destruction, with the exception of hemoglobinuria. This hemoglobinuria without a preceding anemia was difficult to explain. In pigment cirrhosis in humans no anemia is present, and this fact has been utilized by some as an argument against the idea that copper has any effect on hemoglobin, such as hemolysis or destruction of red blood cells. However, the explanation of this phenomenon is doubtless the same as that which applies to our chronically poisoned animals; namely, that the action of copper in small amounts over a long period of time destroys such an insignificant number of erythrocytes each day that it is impossible to detect such a loss. It was not until we carried out our acute poisoning experiments that the action of copper on the blood was revealed.

In such acute poisoning an anemia developing within one to two days after injection is the rule. Coincident with the development of this anemia there appears in the liver a copper-containing compound in the form of pigment granules which morphologically and tinctorially resemble hemofuscin. That such pigmentation is related to the anemia can be shown by comparing the effect of acute poisoning in the guinea pig. In this animal no anemia develops and similarly no deposition of pigment occurs, a fact which would seem to demonstrate beyond question the relation of the action of copper on the blood to the pigmentation of the liver. The occurrence of copper in the pigment granules is a further point of great importance for our contention that such pigment is the result of copper poisoning and is not normal pigment or pigment derived from certain foodstuffs.

The mode of action of copper in acute poisoning would seem to be as follows: the first effect is hemolysis of a certain number of red cells leading to a liberation of hemoglobin. The evidence in favor of this



creted in the bile. At this time the pigment granules stain like hemofuscin, but contain no copper or demonstrable iron. However, at a later period, unmasked iron begins to appear and the pigment takes on the characteristics of hemosiderin. In addition to causing the deposition of pigment, the copper leads to degeneration and necrosis of the liver cells, and, if continued over a long enough time, a form of pigment cirrhosis results.

It should be noted that there is a variation in the reaction of different species of animals to copper poisoning. The monkey (*macacus rhesus*) requires a larger dose of copper than the rabbit, but the lesions produced are the same. The sheep is very susceptible and death from hemoglobinuria is likely to occur if the dose is too large. Pigmentation in the liver is not so prominent, but necrosis, cirrhosis and bile stasis are conspicuous. The guinea pig has been found very resistant to the action of copper except as regards the kidney, where a tubular nephritis commonly occurs. It has not been possible to produce an anemia, and pigmentation occurs only after a long continued series of injections of copper.

### SUMMARY AND CONCLUSIONS

1. Acute poisoning with copper causes anemia, hemoglobinuria, necrosis of hepatic and renal cells and pigmentation.

2. The pigment so formed is a combination of copper and some derivative of hemoglobin and can be stained with a neutral solution of hematoxylin.

3. The differential staining property of this pigment depends on its copper content.

4. The pigment granules which often occur in the rabbit's liver under natural conditions and which certain other investigators have mistaken for those due to the action of copper are not colored by this method.

5. As a result of repeated injections of copper over a long period of time, a form of pigment cirrhosis results.

longer demonstrable, but unmasked iron has begun to appear. The presence of this unmasked iron in the pigment granules is in favor of the supposition that the pigment granules are originally composed in part of some derivative of hemoglobin rather than of one of the bile pigments or porphyrins, as has been suggested.

In our former work we claimed that chronic poisoning with copper led to pigmentation of the liver and to cirrhosis. The pigment so formed gave all the staining reactions of hemofuscin, and in animals that had survived a sufficient period of time iron could be demonstrated, the pigment then having the characteristics of hemosiderin. Our results were denied by some and confirmed by others, and it was apparent to us that several points had to be investigated in order to establish firmly our claims.

One of the difficulties was the question of the relationship of the pigment in normal rabbit livers to that which we believed to be caused by copper poisoning. At that time, we had no means of distinguishing the two and their apparent identity quite naturally led to scepticism on the part of other workers. However, the result of the application of the method described above has settled this point beyond dispute; namely, the demonstration that the pigment following copper poisoning contains copper while the normal pigment does not. In this way a clear-cut differentiation of the two pigments has been made possible, and, equally important, it has shown the actual presence of the metal in the pigment so formed.

Another point needing elucidation to confirm our conception of the action of copper was the question of the effect of this metal on the blood. This has been discussed above. A final question was whether copper could injure the liver cells and produce cirrhosis. We have found that copper does cause necrosis of liver cells and if this effect is repeated over a sufficiently long period of time cirrhosis results.

In brief, our conception of the action of copper in experimental poisoning is as follows: copper causes hemolysis and a part of the hemoglobin so liberated is taken up by the liver. At the same time copper is absorbed by the liver and there is deposited in the liver cells a compound made up of the two substances in the form of a pigment, staining like hemofuscin and containing demonstrable copper. As time goes on, this pigment increases in amount, but after a period of several weeks following injection of the metal the copper gradually disappears from the pigment granules, presumably being ex-

## DESCRIPTION OF PLATES

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### PLATE 64

FIG. 1. Liver of rabbit (Z 690) injected subcutaneously with 2 cc. of a 20 per cent suspension of powdered metallic copper in lard and killed on the fourteenth day. Numerous pigment granules present in liver cells and to some extent in macrophages. Granules colored deep blue after section was stained for one hour with neutral aqueous solution of hematoxylin.  $\times 500$ .

FIG. 2. Liver of rabbit (Z 649) injected subcutaneously with 6 cc. of a 20 per cent suspension of powdered metallic copper in lard. Animal dead at end of eight weeks. Section shows numerous pigment granules in liver cells and especially in macrophages, stained deep blue by the phloxine-methylene blue method. Beginning cirrhosis evident.  $\times 100$ .

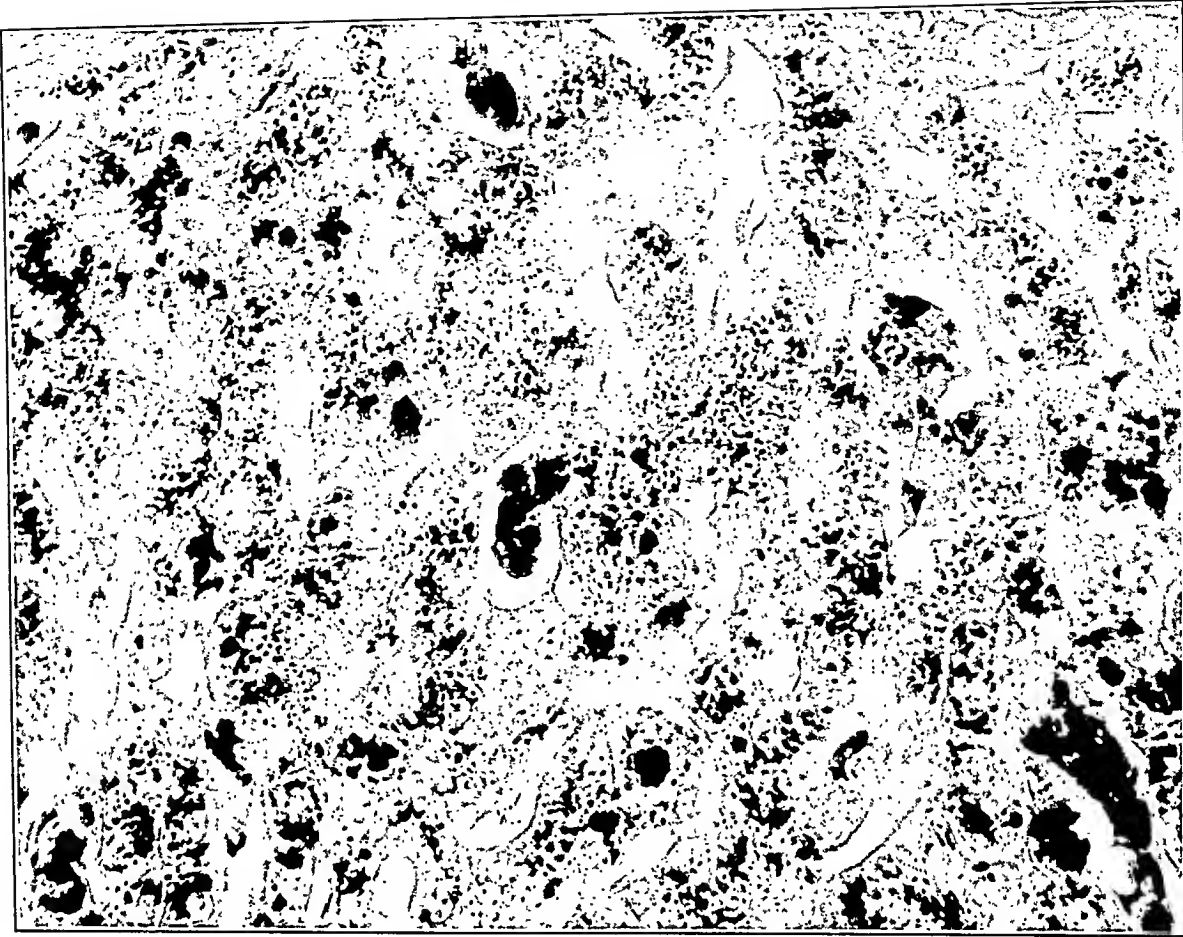
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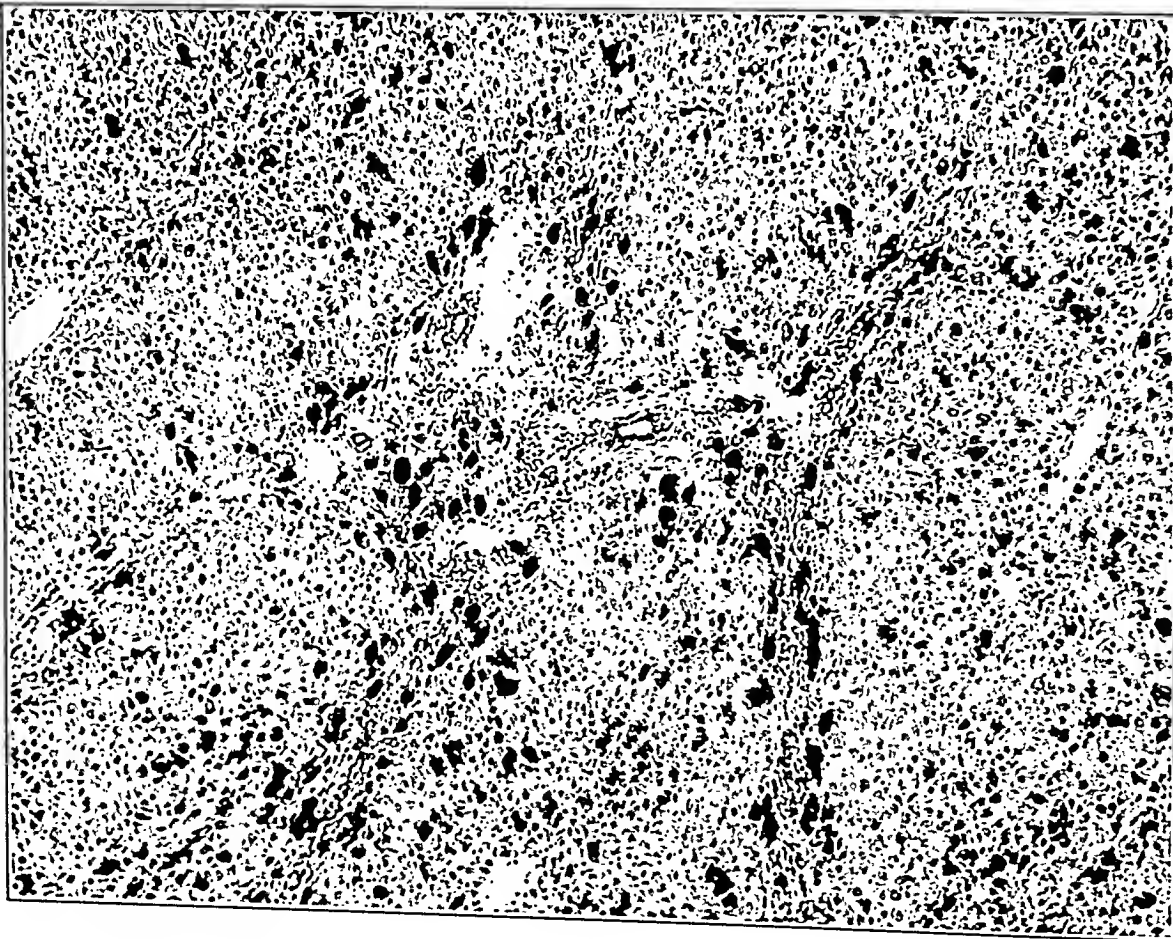
PLATE 65

FIG. 3. Liver of rabbit (Z 211) given copper acetate in moderate doses on food for three years and two months. Biopsy showed fairly numerous rather coarse pigment granules in liver cells at peripheries of lobules; otherwise negative. Metallic copper powder sprinkled on food for following nine months, when animal died. Liver large, very dark (greenish chocolate color); surface finely granular; consistence dense. Section shows pigmentation and marked cirrhosis. Phloxine-methylene blue stain.  $\times 40$ .

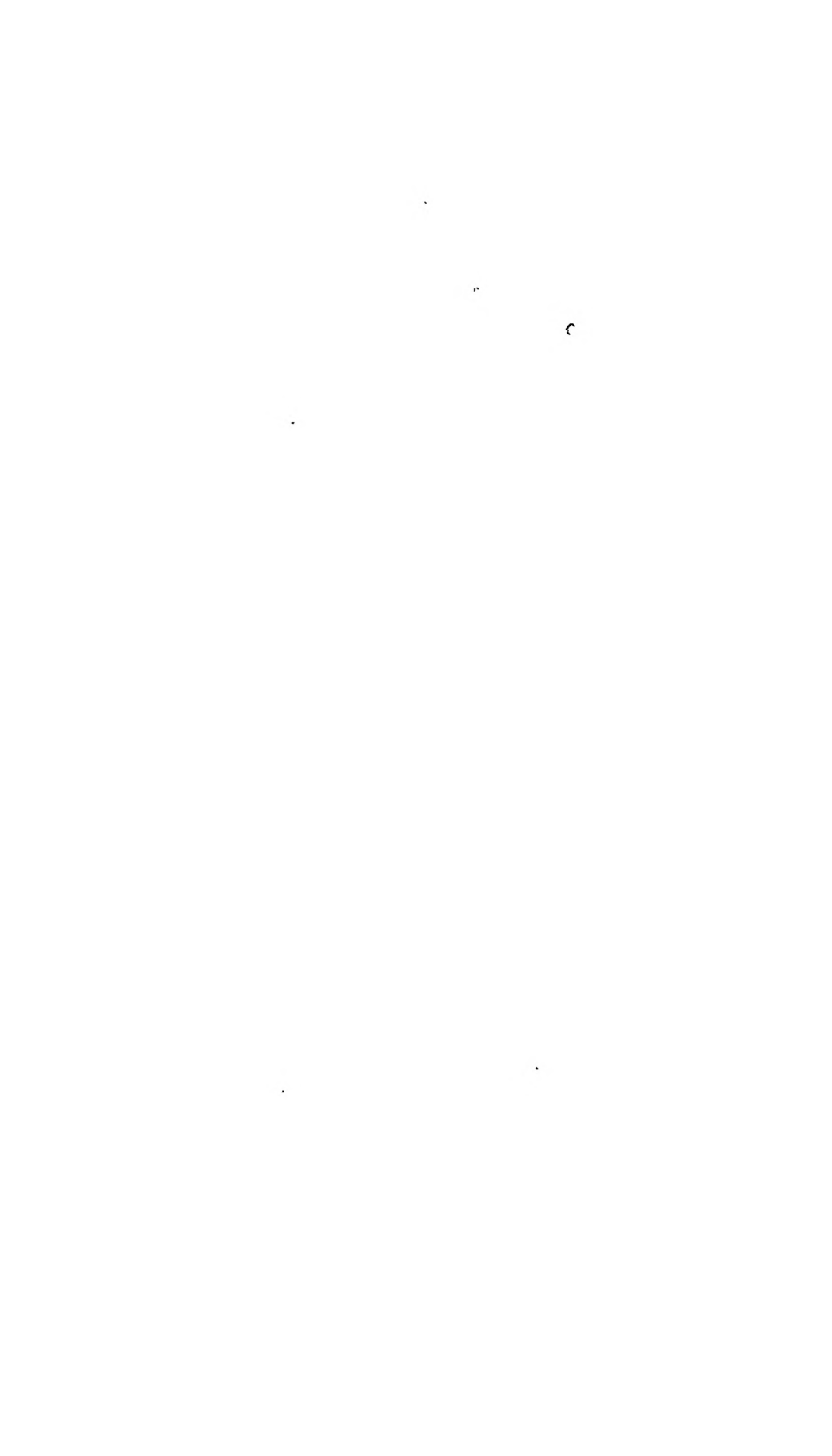
FIG. 4. Kidney of sheep (Z 282) fed copper acetate in moderate doses on food for eleven and a third months. Killed because off feed for over a week. Kidneys large and black. Section shows great numbers of hemoglobin casts in collecting tubules. Phloxine-methylene blue stain.  $\times 100$ .



1

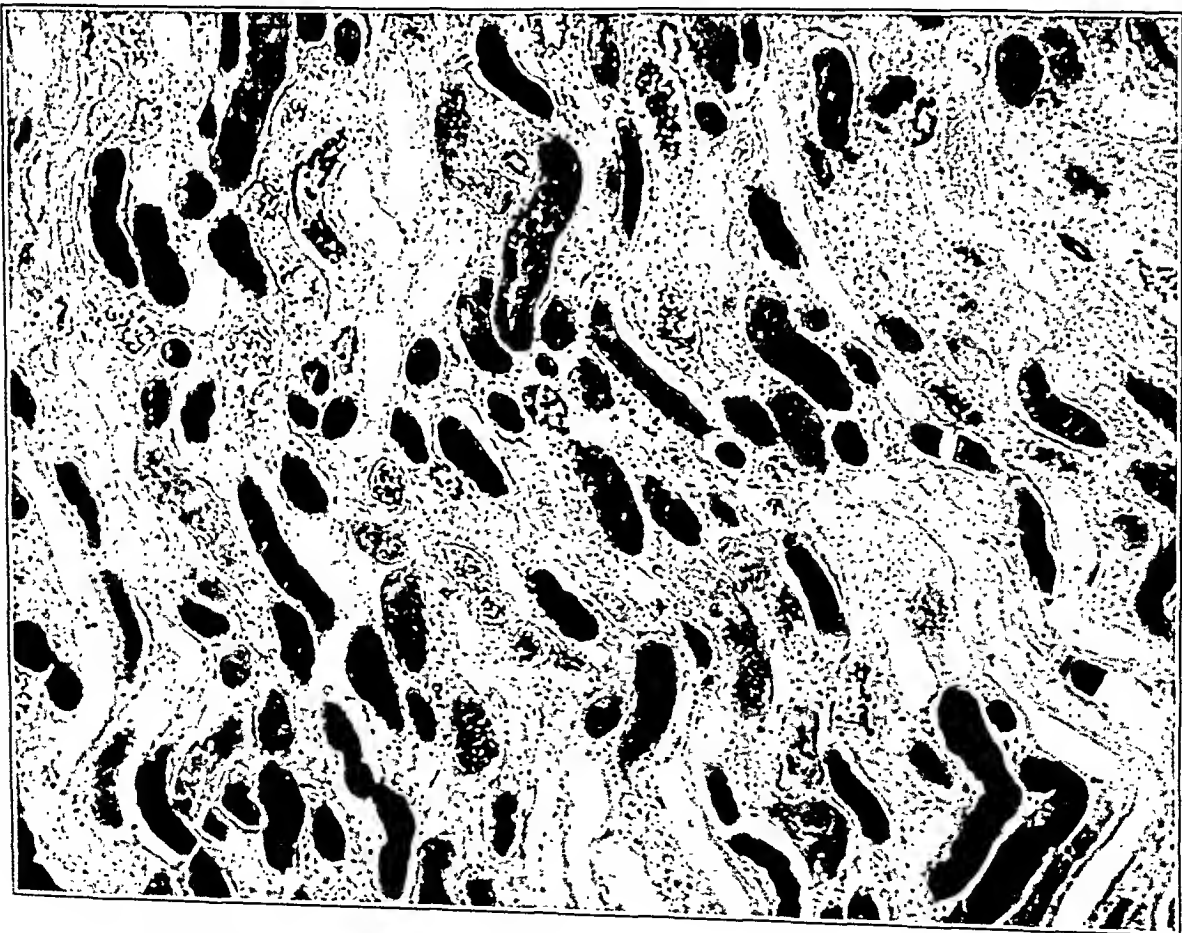


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4



poisoned with copper led to the application of the same methods to the livers from human cases of pigment cirrhosis. The hematoxylin test was found to be much the more useful. Certain points must, however, be borne in mind in the application of the method. Copper is easily removed from tissues by the action of acids and alkalies even if they are very dilute. On this account alcohol would seem to be the best fixative. But after alcohol fixation hematoxylin stains hemosiderin brownish black to black while the copper in the pigment granules is colored light to dark blue. As a result the two kinds of pigment granules may be stained so much alike that they cannot always be distinguished from each other with certainty.

After fixation in formalin or in Kaiserling I, hematoxylin stains hemosiderin yellow to brown (brownish black in the macrophages) while the copper is colored blue, as after alcohol fixation. The reducing action of formalin therefore renders possible a clear differentiation of the two metals. The difficulty is to prevent the disappearance of the copper owing to the acidity which always develops in formalin. Perhaps the best method is to fix in as neutral formalin as possible for 1 to 3 days, wash in running water for 24 hours and preserve in 80 per cent alcohol. Probably neutral buffered formalin could be used to advantage but this method has not yet been tried.

Fortunately copper can often be demonstrated, at least in the coarser pigment granules containing it, even after the tissues have been preserved in formalin for a long time, although it cannot always be stained so intensely as could be desired.

The hematoxylin staining method has already been given in detail. It may be repeated briefly as it should be applied to pigment cirrhosis material obtained in the future.

1. Fix thin slices of liver tissue in neutral buffered (pH 7.0) 10 per cent formalin for 1 to 3 days. Wash in running water for 24 hours. Preserve in 80 per cent alcohol.

2. Stain frozen, celloidin or paraffin sections in a freshly prepared neutral buffered (pH 7.0) approximately 0.5 per cent aqueous solution of hematoxylin for about 1 hour.

3. Wash in several changes of tap water and then allow to stand in it for 1 hour in order to render the blue color of the copper brighter.

4. Alcohol, oil of origanum (cretic) or xylol, xylol balsam.

Copper in the pigment granules and in inspissated bile is stained

## THE MICROCHEMICAL DEMONSTRATION OF COPPER IN PIGMENT CIRRHOSIS\*

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It was suggested in 1921 by Mallory, Parker and Nye<sup>1</sup> that hemochromatosis might be due to chronic poisoning with copper. The idea was based on experimental work which showed that pigment cirrhosis could be produced in rabbits and sheep by adding copper to their food over a long period of time.

The suggestion was urged again by Mallory<sup>2,3</sup> in 1925 and 1926 on the basis of further experimental work and of the study of ten new cases of hemochromatosis which had come to postmortem examination within one year at the Boston City Hospital. Particular attention was paid to indulgence in alcoholic beverages and to occupations. Examination of a number of bootleg liquors for copper demonstrated it to be present in varying amounts and occasionally in considerable quantity in about 10 per cent of the samples tested. Occupations involving exposure to inhalation or ingestion of metallic copper or of its salts was shown to play a possible part in causing chronic poisoning in a certain number of instances. One man had worked for fourteen years in a shop "milling and planing copper and brass."

One of the conclusions drawn in the first paper published was the following: "Proof that hemochromatosis is due to poisoning with copper would require the demonstration of copper either in hemofuscin, or in the liver in excess of the minute amount said to be normally present, or in excretions from the body."

It is believed that all three of these requirements have been fulfilled by the work described in this paper.

The discovery by Mallory and Parker that it was easily possible by means of two delicate microchemical methods to demonstrate copper in sections of the livers of rabbits and other animals acutely

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copper is present in the islands of regeneration. Moreover it was possible by means of the triple nitrite method to verify these results in two out of the four cases tested, as it showed copper in the sections, although not its exact location, while sections of control normal livers failed to do so.

## DISCUSSION

Owing in part at least to our suggestion ten years ago that hemochromatosis might be due to chronic poisoning with copper, much chemical work has been done during the past three or four years by Schönheimer and his coworkers on the question of an increase above the normal in the amount of copper in the liver in pigment cirrhosis.

In 1928 Schönheimer and Oshima<sup>4</sup> reported the examination of livers from seventeen cases of hemochromatosis and stated that they had demonstrated an average increase in the amount of copper of three to four times the normal. Herkel,<sup>5</sup> continuing their work, reported the examination of twenty-four additional cases. He found up to ten times the normal amount of copper. However, he examined also ten cases of non-pigmented cirrhosis. Two contained normal amounts of copper but the other eight showed a marked increase, in part exceeding that present in pigment cirrhosis. As a result of his chemical examinations he did not feel that he was justified in holding copper responsible for causing hemochromatosis.

By means of two very delicate chemical tests we have been able to demonstrate copper in the islands of regeneration in five cases of active pigment cirrhosis. It occurs in the pigment granules of the young regenerating liver cells and also in the masses of inspissated bile occasionally present as the result of focal bile stasis.

The study of the lesions in these five cases demonstrates clearly that copper is deposited in the young liver cells and is bound up with a derivative of hemoglobin with which it forms yellow pigment granules (copper hemofuscin). In the course of weeks to months the copper disappears, apparently in the bile in which it is regularly present in demonstrable quantity, and leaves behind pigment granules which for a time may give no reaction for iron and are called hemofuscin. Both types of hemofuscin granules stain deeply with basic aniline dyes. Later these pigment granules undergo a chemical change as a result of which they react for iron (hemosiderin). Some-

light to dark blue; iron (hemosiderin) yellowish brown to brownish black.

In examining pigment cirrhosis livers for the presence of copper it is necessary to select the most active cases because otherwise all the copper may have disappeared. What is wanted are livers which contain numerous islands of regeneration, because it is only in young liver cells just pigmented that copper can be found. We have studied five livers which fulfil these conditions and found copper present in all of them.

The best preservation of copper was in the most recent case, probably because the acid in the formalin had had little time to dissolve out the metal, although it may have been due to the fact that at times we have attempted to neutralize the fixative. One of the five cases dated back to 1917 and it was still possible to demonstrate copper in the coarser pigment granules. In two of the more recent cases it was impossible to demonstrate copper after formalin fixation, probably owing to marked acidity, but easy after alcohol fixation.

The findings in the five different cases of pigment cirrhosis were much alike so that they may be considered together. The islands of regeneration were of all sizes, from a few cells up to areas 2 and 3 mm. in diameter and of various ages. Some contained no pigment granules, others many, both fine and coarse. Practically all of the regenerating foci had a distinct bluish tint, suggesting that copper was present in the cytoplasm of the liver cells as well as in the pigment granules. As the cells in the foci aged and the copper was dissolved out the pigment granules either appeared yellow, taking no stain from the hematoxylin, or showed all gradations from blue to black owing to the development of hemosiderin while more or less copper was still present. The presence of the iron pigment could be demonstrated also by means of the ferrocyanide of potassium and hydrochloric acid reaction.

In a few of the islands of regeneration and less often elsewhere small masses of inspissated bile were present owing to obstruction to its outflow. These masses stained light to dark blue as a result evidently of the presence of copper in them because ordinary inspissated bile is not stained by hematoxylin.

These five cases of pigment cirrhosis demonstrate therefore beyond question, as the result of the differential hematoxylin stain, that

siderable amount in the liver in pigment cirrhosis reasonably suggests that it is the cause of the lesion, because it has been shown that this metal when introduced into rabbits and other animals in sufficient quantity leads to marked pigmentation and to necrosis of liver cells, terminating in cirrhosis. In favor of this view is the fact that necrosis is more marked centrally, pigmentation peripherally in the lobule.

### SUMMARY AND CONCLUSIONS

1. The presence of copper was demonstrated in islands of regeneration in five active cases of pigment cirrhosis by means of the hematoxylin test. Its presence in the sections was confirmed by the triple nitrite test in two of the four cases tested.

2. The copper occurred in pigment granules in the young liver cells and in masses of inspissated bile.

3. After causing the deposition of a copper hemoglobin compound the copper is quickly eliminated in the bile and therefore does not accumulate in the liver.

4. The pigments (copper-hemofuscin, hemofuscin and hemosiderin), derived successively from hemoglobin and containing masked and unmasked iron, require years to transform them. As a result they accumulate in large amounts in the liver and form the most prominent feature of this type of cirrhosis.

5. The necrosis of liver cells, which eventually results in cirrhosis, is apparently due to the toxic action of copper and not to the mechanical presence of the pigments.

6. Proof has been presented that copper is present in the early hemofuscin pigment granules, in the excretion, bile, and also in excess of normal in the liver tissue, as evidence that chronic poisoning with copper causes hemochromatosis.

times this change takes place rather quickly while more or less copper is still present in the granules.

The quick elimination of the copper is supported by our work on acute copper poisoning in rabbits and other animals in which we showed that the copper had practically disappeared from the liver within five months after subcutaneous administration of it had ceased.

The fact that so much more iron than copper is present in the liver in hemochromatosis is difficult at first to understand. From the evidence presented the explanation is probably simple: the copper is quickly and steadily eliminated in the bile. The iron in the hemoglobin derivatives remains behind and accumulates for years. Apparently it cannot be eliminated until hemofuscin breaks down to hemosiderin. Then it very slowly dissolves and disappears. The injurious action of the copper is in part due to its combining with hemoglobin and causing it to be deposited as copper hemofuscin in the liver and other organs.

The presence of considerable copper in non-pigmented cirrheses may be due to the retention in the bile of the copper normally eliminated in it. Focal bile stasis as the result of cirrhosis would lead to the accumulation of the normal amount of copper passing through the liver.

Owing to the ready solubility of copper in acids and alkalies it seems evident that chemists will not obtain the correct amount of this metal present in livers in hemochromatosis until they use dried fresh material or preserved material plus all the fluid in which it was fixed.

The presence of copper in the inspissated bile in these cases of pigment cirrhosis is of interest in view of the recent work by Schönheim and Herkel.<sup>6</sup> They have shown that pigment gall-stones contain copper in large amounts up to 10,000 mg. per kilo, far more than is present in any organ or tissue in the body. Their observations and ours indicate clearly that copper is eliminated through the bile and therefore through the gastro-intestinal tract.

The cause of necrosis of the liver cells which finally leads to the formation of pigment cirrhosis has always been a puzzle. An equal amount of pigment in the liver cells in pernicious anemia produces no such effect. The demonstration that copper occurs focally in con-

## DESCRIPTION OF PLATE

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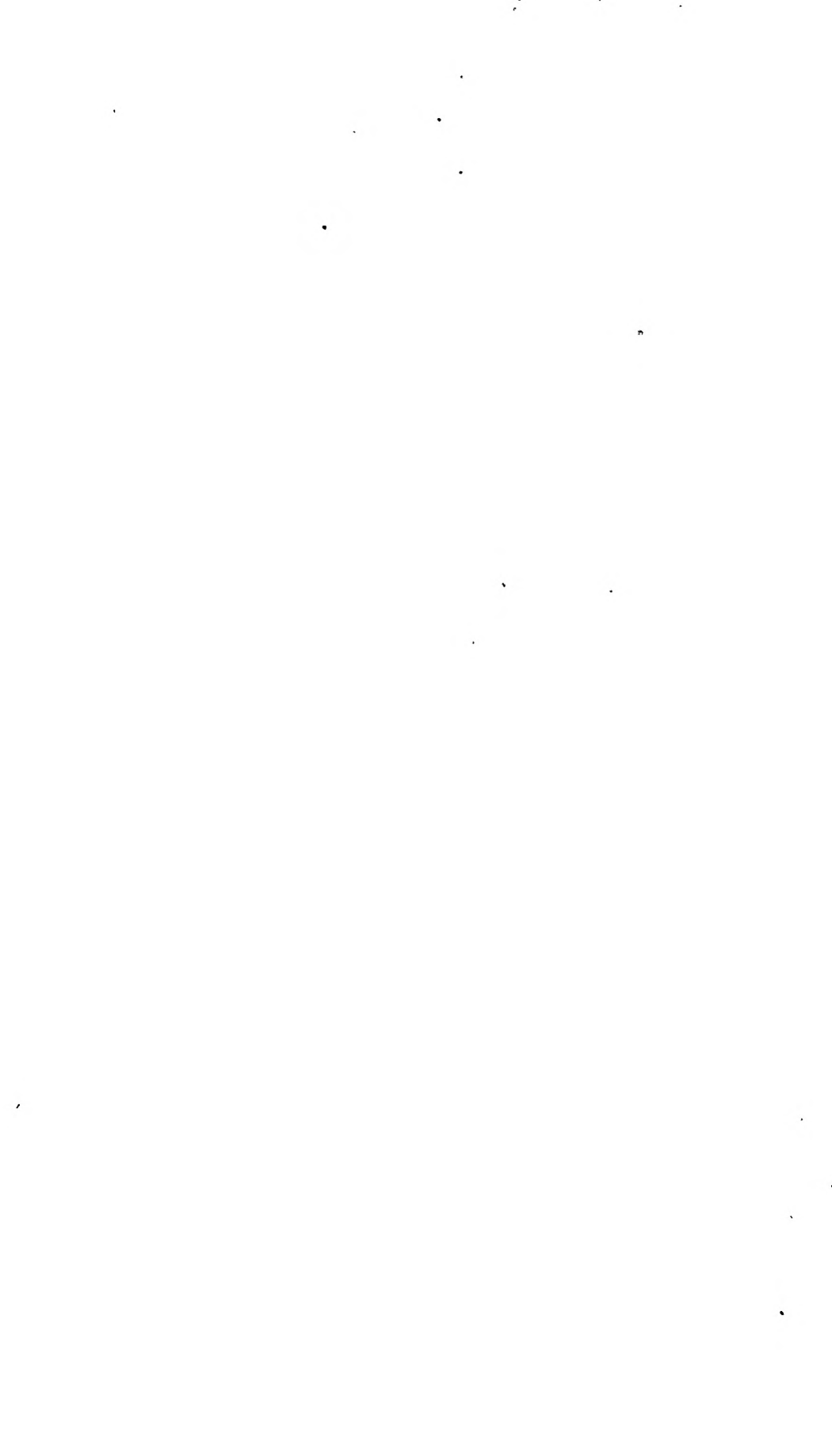
### PLATE 66

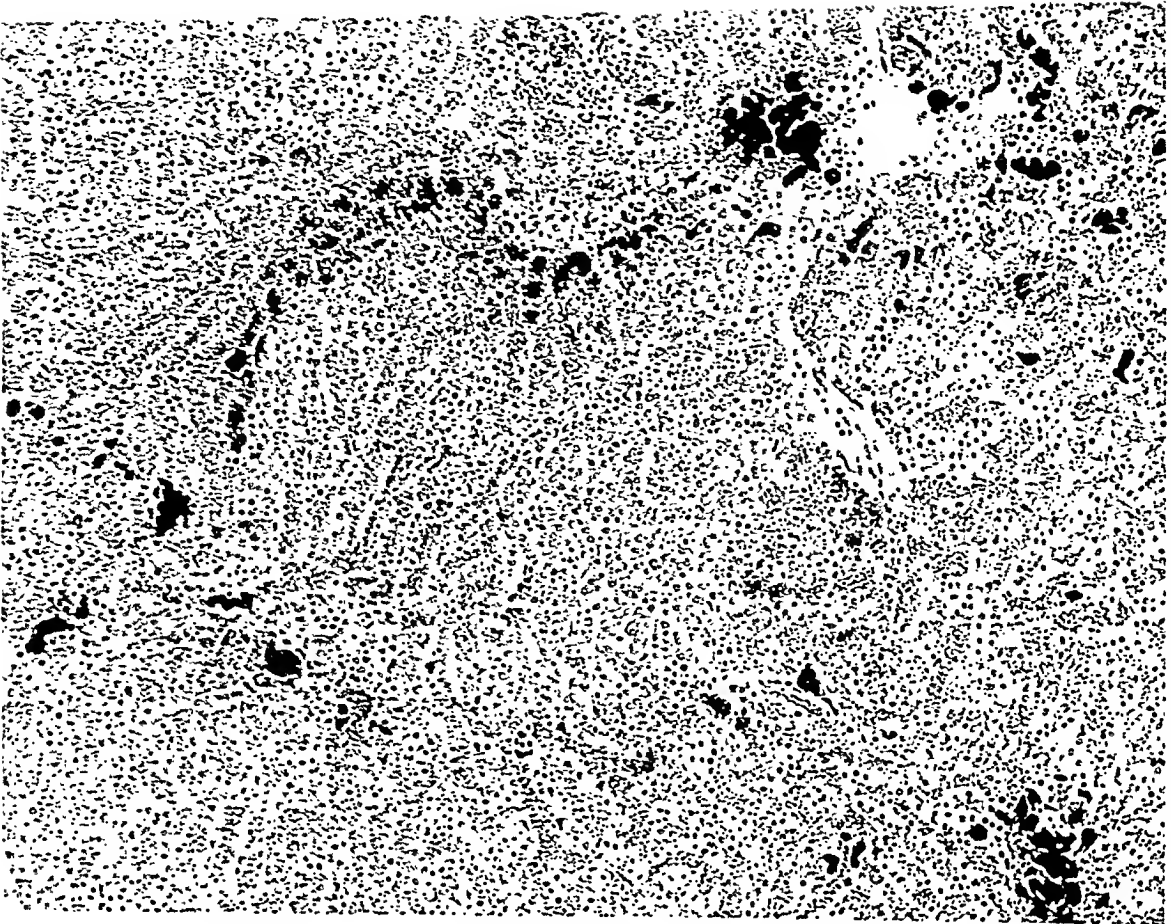
- FIG. 1. Pigment cirrhosis (S 31-218). Kaiserling fixation. Section stained for 1 hour in neutral aqueous solution of hematoxylin. Pigment granules in liver cells in an area of regeneration colored blue, hemosiderin granules in adjoining liver cells and macrophages yellowish brown to black.
- FIG. 2. Pigment cirrhosis (S 31-218). Fixation and staining as in Fig. 1. Pigment granules in regenerated liver cells and in masses of inspissated bile colored deep blue.
- FIG. 3. Liver of rabbit (Z 690) injected subcutaneously with 2 cc. of a 20 per cent suspension of powdered metallic copper in lard and killed at the end of fourteen days. Pigment granules in liver cells and macrophages stained deep blue in 1 hour in a neutral aqueous solution of hematoxylin.

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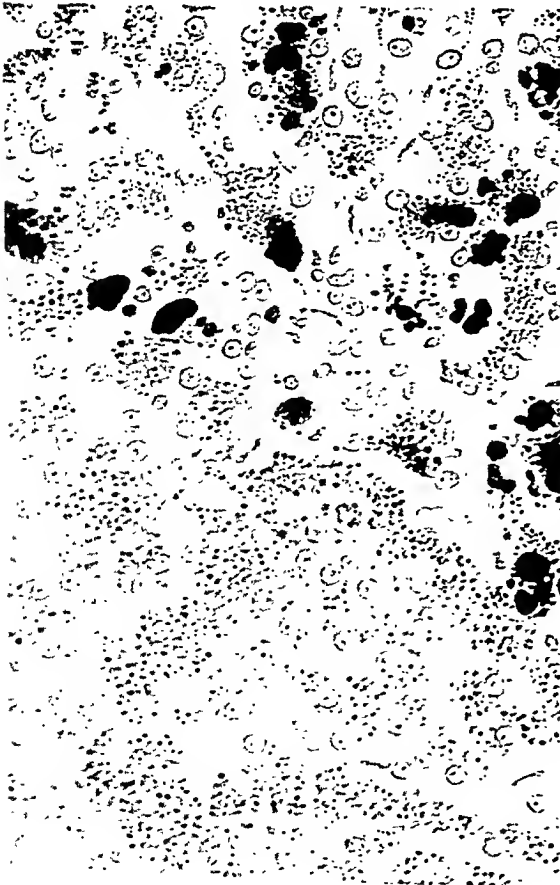
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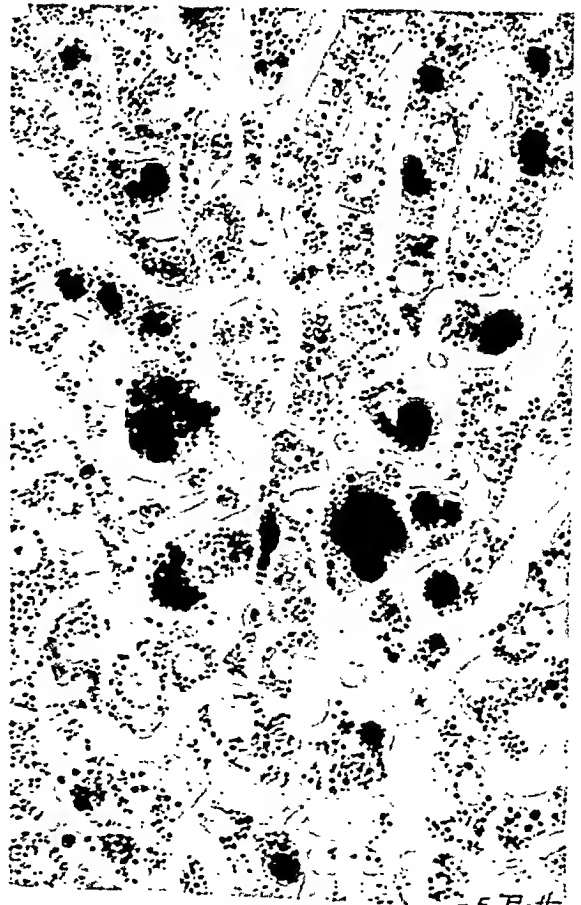




1



2



3

F. Roth

Mallory and Parker

Demonstration of Copper in Pigment Cirrhosis

## PRELIMINARY EXPERIMENTS

No bacteriological studies were made upon the first three birds exhibiting the unusual features just mentioned, but cultures of the blood, liver, spleen, kidney and bone marrow of the fourth bird (Pigeon 86) to die under these conditions all gave pure growths of a small bacillus which was identified by Dr. L. T. Webster of the Rockefeller Institute as *Bacterium aertrycke*.

This organism, which varies from 1 to 2.5 microns in length and from 0.3 to 0.4 of a micron in thickness, is a Gram-negative, motile rod with rounded ends. In smears prepared from young cultures, the bacillus varies in morphology from rod to almost coccoid forms and its body stains evenly, though individual organisms with more deeply staining ends are usually present. Numerous long, peritrichal flagella are revealed by appropriate stains. In cultures, both acid and gas are produced in dextrose, maltose, mannite, levulose, xylose, galactose and dulcitol, the reactions being more marked in the last four sugars than in the three former ones. Saccharose and lactose are not affected, even after several days' incubation. Indol is not formed. Litmus milk is rendered slightly acid within the first twenty-four hours but the reaction later changes to alkaline. Milk is not clotted. On potato the growth is very poor, forming only a faintly visible, grayish white pellicle. Coagulated serum and gelatin are not liquefied. In broth the growth produces even cloudiness, but after several days a grayish white sediment is usually formed. Cultures on lead acetate medium show that  $H_2S$  is produced. On meat infusion agar slant the organism forms a thin, transparent, confluent growth, a characteristic of all bacteria belonging to the paratyphoid group. On blood agar plates the growth is luxuriant; large, round, flat colonies with pale centers are formed in twenty-four hours. On China blue rosolic acid medium the colonies are small, round, slightly elevated and of distinctly pinkish color.

The immunological behavior of this organism strongly suggests that it represents a separate species. Cultures are agglutinated by immune pigeon and rabbit sera in high dilutions, the usual titre being 1:2560. *B. typhosus* and *B. paratyphosus B* are agglutinated by anti-aertrycke serum in dilutions of 1:40 and 1:10 respectively. Rabbit sera rendered immune to *B. typhosus*, *B. paratyphosus A*, *B*, and *C* usually agglutinate *B. aertrycke* in dilutions up to 1:40. The

# SPONTANEOUS AND EXPERIMENTAL INFECTION OF PIGEONS WITH B. AERTRYCKE \*

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## INTRODUCTION

During the course of some experiments upon blood formation in pigeons, which had first been undernourished for varying periods of time and then maintained upon diets consisting mainly of fetal beef tissues, an occasional bird became critically ill, developing diarrhea, grave anemia, and high leucocytosis with many young myeloid cells in the peripheral blood. Death invariably occurred within a few days after the onset of these symptoms. Such pigeons, though greatly emaciated, showed at autopsy marked enlargement of the liver, spleen and kidneys, as well as extreme myeloid hyperplasia of the bone marrow. Throughout the liver and kidneys, innumerable small yellowish gray points and irregularly shaped zones were grossly visible. Upon microscopic examination these areas were found to consist mainly of large numbers of myelocytes grouped about the blood vessels in these organs. The spleen showed a marked increase of large, clear, mononuclear, phagocytic cells and almost complete absence of lymphoid tissue, but contained no such accumulations of myelocytes as were seen in the liver and kidneys.

These changes presented a striking contrast to the fatty bone marrow and atrophied organs of the other equally poorly nourished pigeons of our series dying or killed after identical experimental procedures. Though it appeared that the exceptional pigeons were dying of some unusual complication, possibly of bacterial origin, we were unfamiliar with any variety of microörganism which had been shown to be capable of producing such lesions. Therefore, the changes in the blood and bone marrow, as well as the extensive extramedullary formation of myeloid tissue, seemed to us to present a problem worthy of further study.

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pigeons injected intraperitoneally with 2 cc. of a thick saline emulsion of liver, kidney, or bone marrow, two which had received liver became acutely ill within twenty-four hours and died forty-eight hours and five days respectively after inoculation. Shortly before death one of these birds exhibited a leucocytosis of 75,000 cells per cmm., of which 3 per cent were myelocytes. The other showed only a slight increase in total number of leucocytes, 21,000 per cmm., but of this number 71 per cent were myelocytes. These two pigeons showed acute fibrinopurulent peritonitis, as well as the same types of anatomical changes seen in Pigeon 86, and *B. aertrycke* was recovered from the liver of each of them in pure culture. None of the remaining five birds showed any symptoms of disease following injection, or any macroscopic lesions\* when killed a month later, but *B. aertrycke* was grown from the livers of three of them at this time. Cultures made from the livers of the other two birds were sterile.

Three normal pigeons were injected intraperitoneally with 0.1 cc., 0.5 cc., and 1 cc. respectively of a pure 24 hour broth culture of *B. aertrycke*, originally isolated from the liver of Pigeon 86. The first bird showed no manifestations of disease and no anatomical changes when killed a month later. The other two birds, however, died four days and thirty-six hours respectively after inoculation, showing all of the clinical and most of the anatomical characteristics of the disease under study. In addition, fibrinopurulent peritonitis was present in both instances. No myelocytic infiltrations were found in the liver and kidneys of the bird dying thirty-six hours after inoculation. From the livers of all three pigeons *B. aertrycke* was isolated in pure culture.

It therefore seemed highly probable that infection with *B. aertrycke* was the cause of the unusual lesions and death of certain of our birds but, inasmuch as each pigeon was kept in a separate cage and cultures of their water and food failed to reveal the presence of this organism, the source of the infection was not clear. However, since it had been demonstrated experimentally that small doses of *B. aertrycke* had little or no effect upon normal pigeons, and, moreover, that these bacteria often survived within the body for at least a month without producing symptoms of disease or anatomical

\* In the removal of this work from New York to Peiping the microscopic preparations made from the tissues of these birds were unfortunately lost.

blood of normal pigeons rarely contains agglutinins for *B. aertrycke*, and even on these occasions the bacilli are not agglutinated in dilutions greater than 1:20.

Inasmuch as we have subsequently used the culture of organisms isolated from the liver of Pigeon 86 to produce all experimental infections, we submit the following complete protocol of this bird.

TABLE I  
*Protocol of Pigeon 86*

Diet	Full grain (Apr. 24-26)	Starvation (Apr. 27- May 3)	5 gm. fetal liver daily (May 4-16)						Food refused	
			May 6	7	10	13	14	15	16	
Dates blood examined. . .	April 25	May 2	May 6	7	10	13	14	15	16	
White blood cells (thousands per cmm.)	14	4	11	9	14	55	69	133	187	
Granulocytes %	40	54	78	86	64	87	88	91	85	
Myelocytes %	0	0	0	0	0	0	0	0	5	
Lymphocytes %	42	44	12	4	26	1	5	4	4	
Monocytes %	18	2	10	10	10	12	7	5	6	
Red blood cells (millions per cmm.)	3.7	2.6	3.3	3.1	3.1	2.5	2.2	1.9	1.2	
Thrombocytes (thousands per cmm.)	75	16	25	36	37	26	59	33	37	
Weight (gm.)	425	355	320	320	300	280	265	245	230	Died

The *liver* and *kidneys* were found to be greatly swollen and studded with massive accumulations of myelocytes intermingled with smaller, basophilic, poorly differentiated, mononuclear cells. Many large mononuclear phagocytes and bacteria were found in the sinuses of the liver. The *spleen* was also much enlarged, due mainly to the presence of large, mononuclear phagocytes, but showed no such accumulations of myeloid cells as were seen in the liver and kidneys. The heart, lungs, alimentary tract, reproductive organs and brain were unaltered.

At the time of autopsy the blood as well as emulsions of the various organs of Pigeon 86 were injected into a series of normal birds. Of three pigeons, each injected intraperitoneally with 1 cc. of blood, none developed any symptoms of disease. When these birds were killed for study a month later, no anatomical changes were found, but *B. aertrycke* was grown from the livers of two of them. Of seven

consisting of aggregations of mononuclear phagocytes and polymorphonuclear leucocytes, were readily explained. However, such massive myeloid hyperplasia of the bone marrow, the frequent appearance of large numbers of myelocytes in the peripheral blood, and the striking heterotopic formation of myeloid tissue in the liver and kidneys presented a picture of myeloid activity hitherto unrecorded in the course of bacterial infections. Furthermore, since *B. aertrycke*, in several instances, had been found in pigeons which showed no symptoms or anatomical evidence of active infection with this organism, and also had been shown experimentally to survive in the tissues of pigeons for considerable periods of time without producing disease, it was obvious that further facts must be sought before the relationship of this organism and the interesting lesions with which it was frequently associated in pigeons could be understood.

## METHODS

Each bird, upon entering the laboratory, was placed in a separate cage and observed for a period of two weeks, during which time the weight was followed and the blood examined several times. Only those pigeons which appeared normal during this time were used for experimentation.

Supravital staining with neutral red and Janus green,<sup>1</sup> which facilitates a ready distinction of all the cellular elements of the blood, was used routinely in making the differential cell counts. Striking changes in the blood, such as the appearance of numerous young myeloid forms, were always checked in fixed films. The total counts of the red and white cells were made according to the method of Forkner,<sup>2</sup> which we have found to be thoroughly reliable.

In order that experimental infection might be more nearly comparable to infection naturally acquired, we have always introduced the bacteria by mouth, slowly feeding 1 cc. of a 24 hour pure broth culture, drop by drop, with a small pipette. After infection, the blood was studied at frequent intervals until death or recovery. Autopsies, as well as bacteriological and serological studies, were done promptly after death, an occasional moribund bird being killed in order to secure perfectly fresh tissues.

To test our cultures for the presence of some filterable form of microorganism or other substance which might have been respon-

changes, it did not appear unlikely that certain pigeons naturally harbored minimal numbers of this organism and developed generalized infection when subjected to a state of malnutrition. At this point it is of interest to note that a single previously normal pigeon (No. 94), after a period of eight days' starvation, became unduly ill, totally refused food and died within forty-eight hours, showing all of the lesions accompanying infection with *B. aertrycke*. This organism was recovered in pure culture from the liver.

In order to determine whether or not pigeons dying of malnutrition, but showing no evidence of bacterial infection, ever harbored *B. aertrycke* in their tissues, cultures were made of the livers of twenty such birds which had been kept under experimental conditions identical with those of the pigeons dying of infection with this organism. Sixteen of these cultures showed no growth of bacteria, but from four birds *B. aertrycke* was recovered. Though none of these four pigeons showed changes in the blood before death which would suggest infection with *B. aertrycke*, in two of them the characteristic lesions accompanying infection with this organism were found. The other two showed only the effects of malnutrition.

The results of these preliminary studies may be summarized as follows: In a series of fifty-two pigeons, which first had been subjected to a period of starvation and then maintained for varying periods of time on fetal beef tissues, seven cases of an acute, fatal disease associated with marked anemia and enormous activity of myeloid cells occurred. From three of these birds, which were studied bacteriologically, *B. aertrycke* was isolated in pure culture, and in the tissues of the other four an abundance of bacteria morphologically identical with this organism were demonstrated. Four additional instances of the presence of *B. aertrycke* were encountered in twenty routine bacteriological examinations of pigeons of this series showing no clinical evidence of bacterial infection. Two of these showed anatomical changes attributable to the infection with *B. aertrycke*, while the other two did not.

Although it was demonstrated that a disease process could be initiated in normal pigeons by inoculation of tissue from an infected bird as well as by inoculation of cultures of *B. aertrycke*, the nature of the lesions, in both the spontaneous and experimental infections, caused us to have some doubt as to their exclusive relationship to this organism. The lesions in which the bacteria were found,



trated, though Forkner<sup>2</sup> has recently presented a well illustrated study of the blood of normal fowls, which in most essential features resembles that of the pigeon. We have, therefore, devoted most of the accompanying Plate 67, which illustrates supravitality stained cells from the blood of pigeons, to the early myelocytic types encountered in the circulation in our experimental birds.

The granules of the main group of polymorphonuclear leucocytes (Figs. 1 and 2) of the pigeon, as in the fowl, are rod-shaped but exhibit a decidedly more brilliant and darker yellowish red color when stained either supravitality with neutral red or in fixed films with Wright's stain. We have found no cells in the pigeon's blood closely corresponding to the "pseudoeosinophils" found in small numbers in the fowl's blood by Forkner, but we have observed regularly a few myeloid cells, not present in the blood of fowls, with pleomorphic or round nuclei and large, brilliantly eosinophilic granules in their cytoplasm. Such cells, which generally exist only in small numbers, less than 5 per cent, are found to be much more numerous in occasional birds, sometimes comprising 50 per cent of the total number of leucocytes. The relationship of these cells to the other cells of the myeloid series is not clear. They apparently tend to remain high in individual birds which show no other abnormality but, if for any reason a leucocytosis occurs, their numbers have never been observed to increase. We have looked upon such cells as true eosinophils and at first thought that their large numbers were perhaps associated with parasitic infections. Though this association has existed in a few instances, such is frequently not the case. Only recently we have seen an apparently normal pigeon with the duodenum and ileum greatly distended with a solid mass of small nematodes in which the total white cell count was 15,000 per cmm., of which only 2 per cent were eosinophilic myeloid cells with round granules. The granules of the basophils of the pigeon are very small, round and stain deep crimson with neutral red. The monocytes (Figs. 12, 13), lymphocytes (Fig. 14), erythrocytes, and thrombocytes show only very slight variations in morphology from those of the fowl. The general appearance of the myelocytes of the two species is about the same, though slight differences in color of the granules exist in both supravitality stained (Figs. 5 to 11) and fixed preparations.

In the following table are summarized the results of our studies

sible for the excessive growth of myeloid tissue regularly accompanying infection with *B. aertrycke*, normal pigeons were fed or infected with sterile filtrates prepared from this organism.

To serve as controls for our other observations, bacteriological, serological and anatomical studies were made upon a series of apparently normal pigeons.

### THE BLOOD OF NORMAL PIGEONS

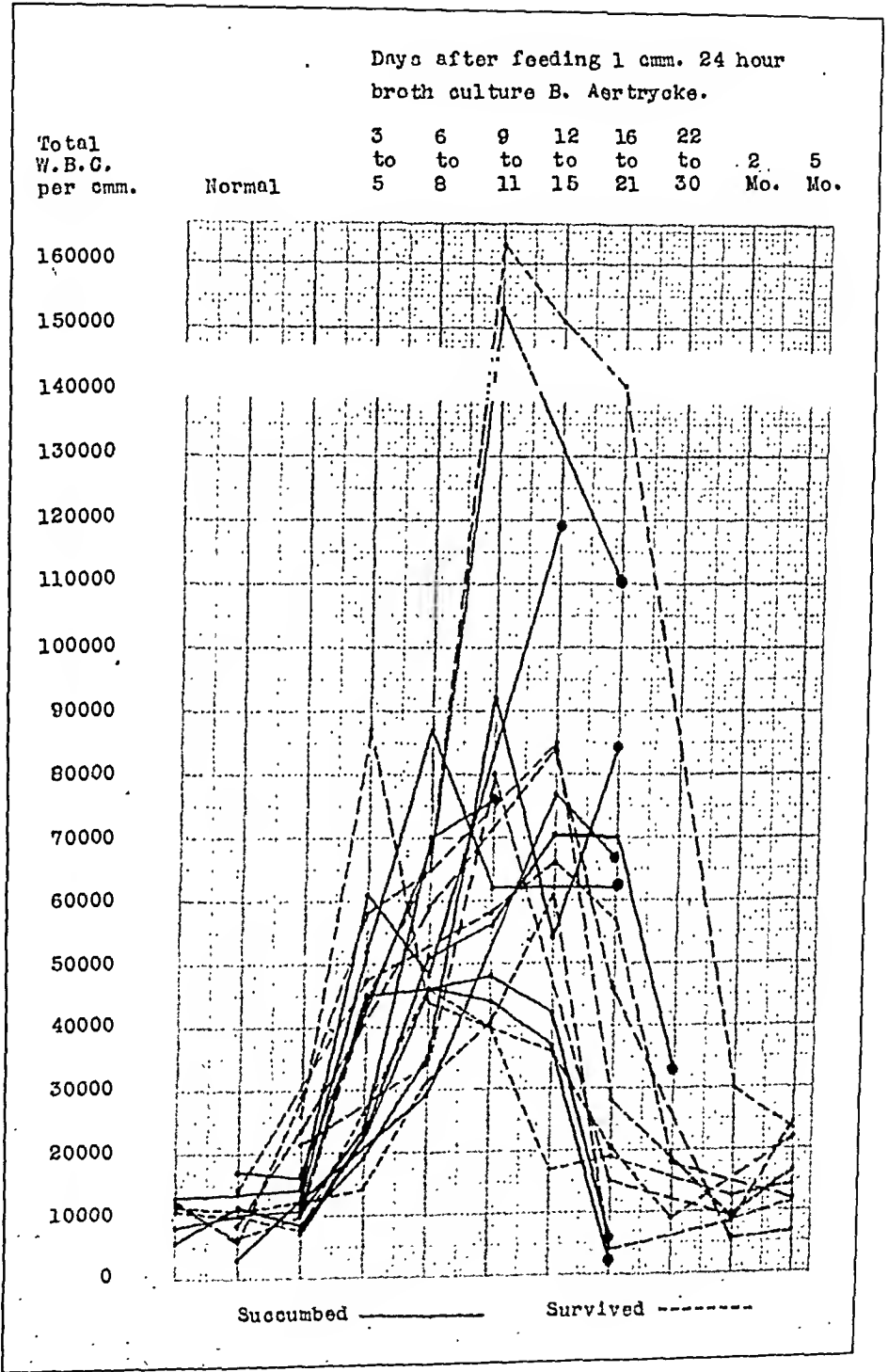
The blood of normal pigeons has been the object of study of only a few investigators,<sup>3, 4, 5</sup> but all of those who have dealt with it have reported marked fluctuations in number of all types of blood cells.

During the course of our experience, based upon 182 apparently healthy pigeons bought at random in the markets of New York and Peiping, we have found that the red blood cells remain unusually constant in individual birds, fluctuations of as much as 400,000 cells being quite exceptional. When first brought into the laboratory, most pigeons show erythrocyte counts of 3,500,000 to 4,000,000 cells per cmm. Only an occasional bird shows a slightly higher count, while erythrocyte counts lower than 3,000,000 almost invariably have been found to be associated with some obvious disease.

The white blood cells, however, have shown considerably greater fluctuations in number than have the erythrocytes. Though the total number of white cells was generally found to lie between 8,000 and 20,000 per cmm., an occasional bird, in which no disease was apparent, was studied which presented a count far above or below these levels. We have seen a few such pigeons with leucocyte counts of 50,000 cells per cmm., for which there was no obvious explanation. However, after being kept in the laboratory for a few days, the white cells in such instances have invariably fallen to normal levels and later on have shown no tendency to rise to their original height.

The percentages of the different varieties of blood cells also have been found to vary greatly in different birds. They have remained quite constant in individuals, though the total white cell counts of these same pigeons have varied as much as 10,000 cells from day to day. White counts made in rapid succession upon the same bird almost always agree closely.

The morphological characteristics of the various elements of the pigeon's blood, as far as we know, are nowhere adequately illus-





is suggestive of the two types of leukemic manifestation, erythro-leucosis and myeloid leucosis, reported as occurring in different fowls inoculated with identical material from an animal dying of a typical myeloid leukemia. That is, with apparently the same underlying stimulus, two different responses may be elicited, depending upon the individual bird. It is not known at present what the nature of the factors governing the individual response may be.

The leucocytosis following infection with *B. aertrycke* was clearly due primarily to an increase in number of the eosinophilic polymorphonuclear leucocytes with rod-shaped granules (Chart 2). Within three to five days after infection mitochondria became visible in the motile granulocytes. The delicately shaped rods of these cells became greatly swollen (Figs. 1, 2, 4), assuming an oval or rounded form though they still stained brilliantly, and atypical microcytes (Fig. 3) were found. After a week many of these younger adult forms of the eosinophilic rod cells, as shown by their slightly indented nuclei and numerous mitochondria, were seen regularly in the peripheral blood. The motility of all granulocytes was always strikingly decreased during the entire course of the infection. Actual myelocytes, frequently very young forms, appeared in practically all pigeons of this group in numbers varying from 2 to 10 per cent of the total number of leucocytes, but in no instance of experimental infection have we observed such a great number of myelocytes in the peripheral blood as frequently occurred in undernourished pigeons dying from infection with *B. aertrycke* naturally acquired. Fig. 7 represents the late myelocytes "C,"<sup>32</sup> with the complement of specific preleucocytic granules nearing the maximum. The myelocytes of the bird have spherical granules throughout the period of maturation in bone marrow, the change to rods occurring coincident with the change in staining reaction, lobing of the nucleus and the acquisition of motility, which immediately precede the extramedullary circulatory appearance and function of these cells. Under normal conditions only fully mature cells with none or very occasional mitochondria, and only rod-shaped granules appear in the circulation. Figs. 5, 8, and 10 represent the stage known as myelocyte "B," midway between myelocyte "C" and the earliest myeloid cells showing specific granulation, namely myelocytes "A" (Figs. 6, 9 and 11). No changes in the polymorphonuclear leucocytes with round eosinophilic granules or in the basophils were noted.

of the blood of normal pigeons. Only birds which were apparently free from disease have been included. The figures given represent average values.

TABLE II  
*Blood Counts on Normal Pigeons*

	New York		Peiping	Total
Number of Pigeons .....	100		82	182
Number of Blood Counts .....	238		139	377

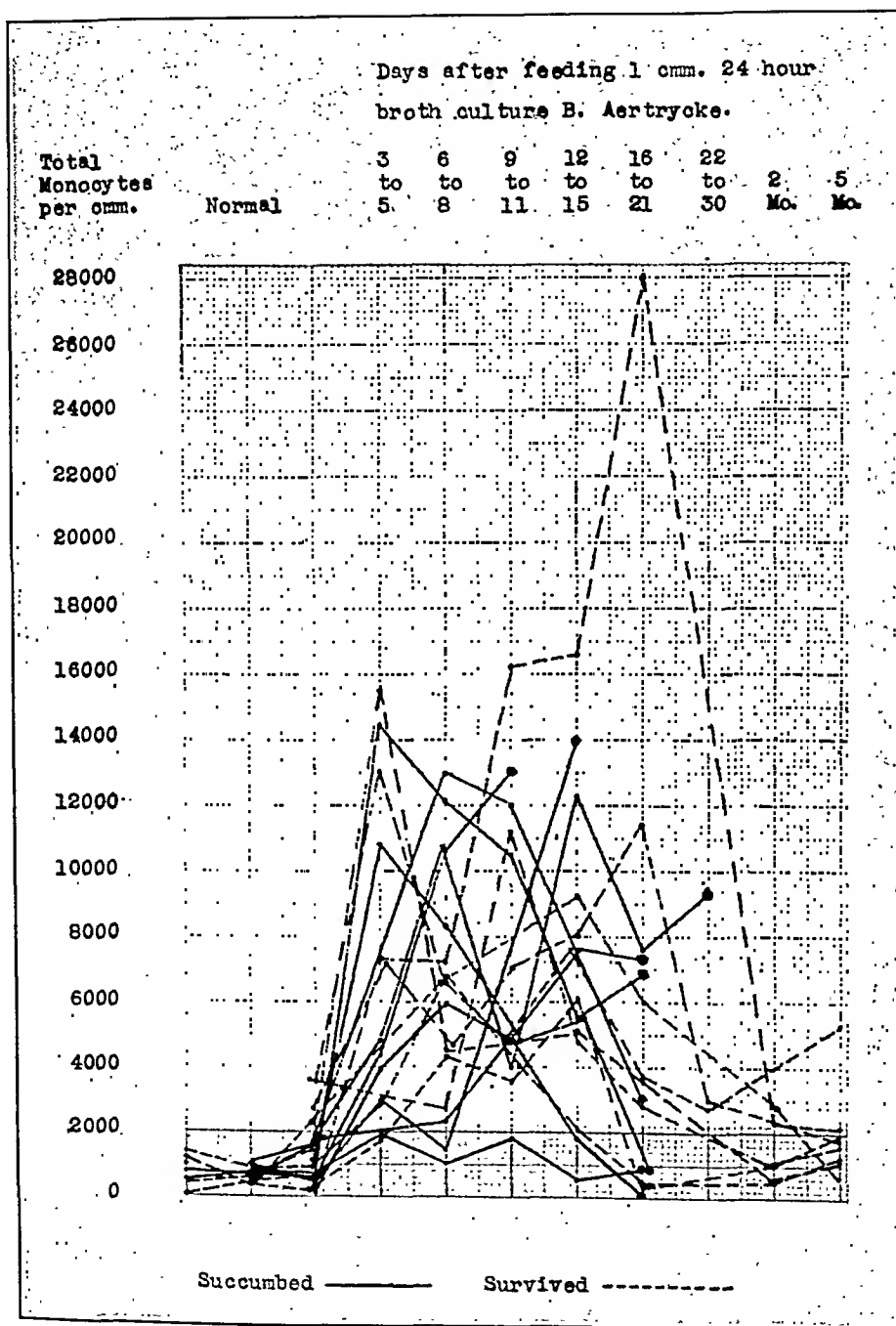
	New York		Peiping	
	Total cells per cmm.	Percentage	Total cells per cmm.	Percentage
Erythrocytes .....	3,813,000	..	3,803,000	..
Leucocytes .....	13,100	..	15,200	..
Thrombocytes .....	34,060	..	42,520	..
Eosinophilic leucocytes with rods ...	5,371	41	5,320	35
Eosinophilic leucocytes with round granules .....	917	7	760	5
Basophilic leucocytes .....	393	3	304	2
Lymphocytes .....	5,109	39	7,144	47
Monocytes .....	1,310	10	1,672	11

#### THE EXPERIMENTAL INFECTION OF PIGEONS WITH *B. AERTRYCKE*

After two weeks preliminary observation, during which time their blood was studied, seventeen apparently normal pigeons were each fed 1 cc. of a 24 hour broth culture of *B. aertrycke*. All of these pigeons became acutely ill within thirty-six hours, most of them developing diarrhea and showing loss of weight, although given food and water in unlimited amounts. Nine of them died within ten to twenty-five days after infection; the remaining eight recovered.

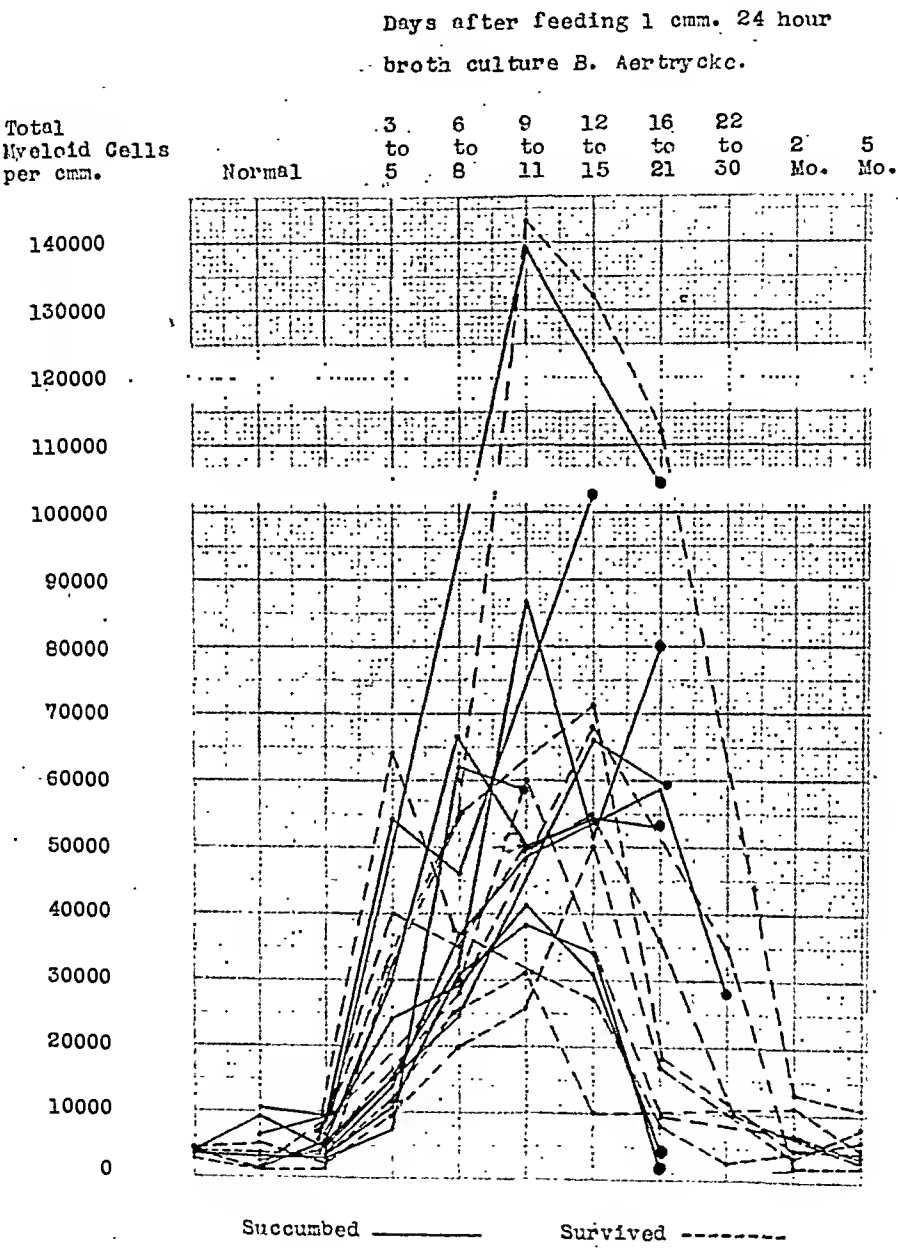
*Changes in the Blood:* After feeding *B. aertrycke* the total number of leucocytes, which had varied in different birds under normal conditions from 6,000 to 29,000 cells per cmm., rose sharply to levels of 46,000 to 160,000 cells per cmm. (Chart 1). Most of the pigeons which died succumbed at a point near the peak of their leucocytosis, but in two birds, after only moderately high leucocytoses of 46,000 and 48,000 cells per cmm., the total number of white cells suddenly fell to 2,000 and 6,000 cells per cmm. respectively. Examination of the bone marrow of these two birds after death showed very few myeloid forms but did reveal a striking degree of hyperplasia of the erythrocytic cells. Extensive myeloid infiltration was present, however, in kidneys and liver of each bird. This particular observation











larly present which closely correspond to the lesions seen in the liver (Fig. 20). In some instances there are also small collections of mononuclear, non-granular cells scattered throughout the interstitial tissue of the kidneys. The epithelium of the tubules is generally much swollen.

Of all the changes occurring in pigeons after infection with *B. aertrycke* those found in the *bone marrow* are perhaps the most extensive. The radius, ulna and femur, which were examined regularly, were filled with solid, firm masses of grayish red, opaque marrow, which literally burst forth from the cracks in the shaft during the process of removal. On microscopic examination one sees a solid mass of myeloid cells in which young forms predominate. The capillaries are collapsed by pressure of the proliferating myelocytes and erythrocytes, so conspicuous in the marrow of normal pigeons, is seen only in certain very limited areas (Figs. 21, 22). Scattered throughout the marrow are found in many instances large and small aggregations of pale, mononuclear cells similar to those seen in the liver. Occasionally, bacteria may be demonstrated in these areas. Capillaries filled with fresh thrombi and bacteria are seen frequently.

The *spleen* is enlarged from one to three times its normal size, is of very soft consistency and pale pink or yellowish brown in color. Microscopically, the most striking alteration is found to be a marked proliferation of large, clear mononuclear cells. This change, which takes place in the follicles as well as the pulp, is attended with almost total disappearance from the entire spleen of cells which can be identified as lymphocytes. Within the sinuses and throughout the pulp many such phagocytic cells, frequently grouped in nodules with a few polymorphonuclear leucocytes about cellular debris and bacteria, are present. Though fairly numerous polymorphonuclear leucocytes and, in a few instances, occasional myelocytes are seen scattered throughout the spleen, no collections of myeloid tissue occur in the spleen which are in any way comparable to those seen regularly in the liver and kidneys. In the case of only one pigeon, which was shown to be ill with acute *B. aertrycke* infection when brought into the laboratory, were large areas of young myelocytes found in the spleen.

The *lungs*, in most instances where infection has been induced by mouth, have shown lobular pneumonia of an unusual type. Situated only in the dependent (anterior) portion of the lungs, about bronchi

The monocytes showed considerable increase in total number after infection with *B. aertrycke*, tending to reach their highest point before the fifteenth day. Many young monocytes with dense, homogeneous cytoplasm and numerous mitochondria appeared regularly during the infection (Chart 3).

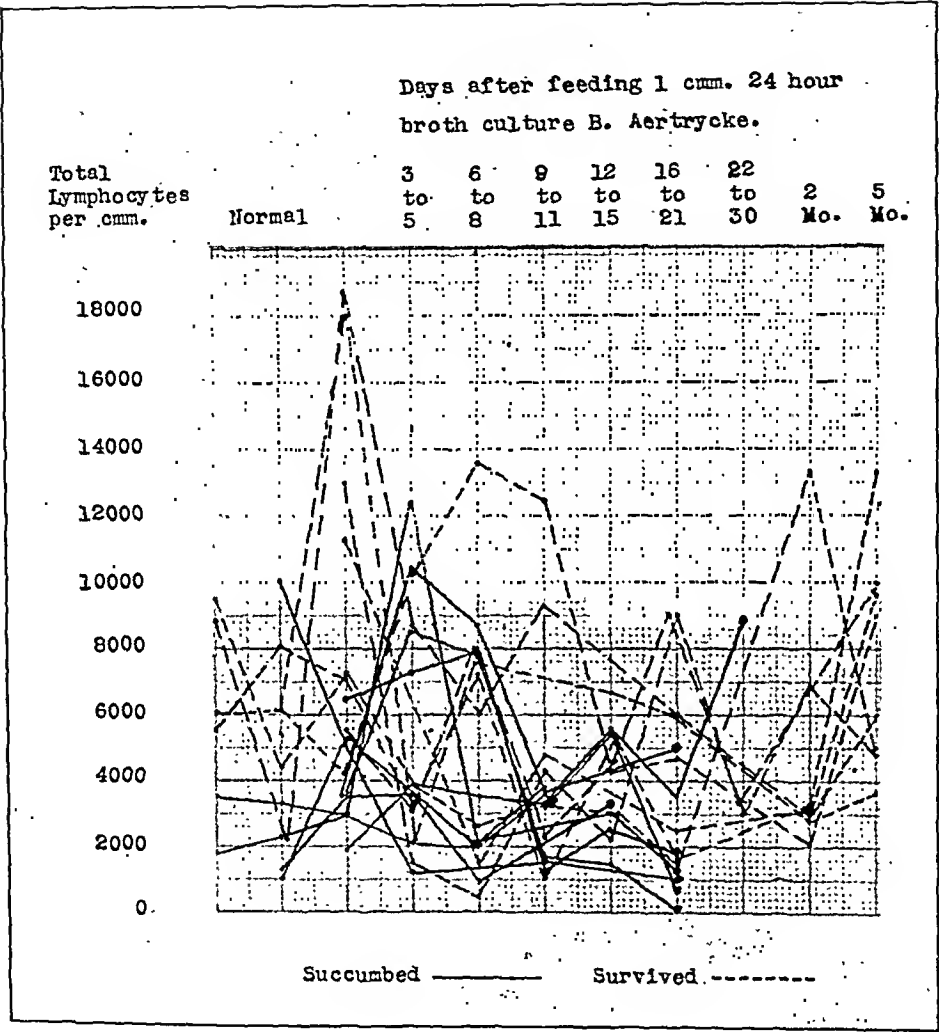
The lymphocytes exhibited a general tendency to fall during the course of the infection. Especially in those birds which succumbed, the diminution of lymphocytes was most marked (Chart 4).

*Changes in the Viscera:* The changes observed in the viscera of pigeons following oral administration of a large dose of *B. aertrycke* have been almost identical in all birds dying as the result of infection. Therefore, since individual birds showed only minor variations in the degree of lesions commonly present, these changes will be discussed collectively. By far the most interesting are those of the liver, kidney, bone marrow and spleen.

The *liver* is always enlarged, at times reaching a weight of 20 gm. It has an opaque, swollen appearance, is generally of pale, brownish red color, and throughout the organ small, round or irregularly shaped patches of opaque, yellowish gray tissue are seen. Microscopically, the liver cells are found to be greatly swollen and the sinuses filled with large, pale, phagocytic cells containing cellular debris, hemosiderin and occasionally red blood cells. Such phagocytic cells, together with a few polymorphonuclear leucocytes, are often found in solid masses, showing varying degrees of necrosis in their central areas where clumps of small Gram-negative bacilli are usually present (Figs. 15, 16). Many such bacilli are scattered in smaller number throughout the liver sinuses. In addition to these lesions, which clearly seem to be due directly to the bacteria which are found within them, each portal and hepatic vein is found to be surrounded by varying numbers of myelocytes intermingled with lesser numbers of slightly smaller, basophilic, mononuclear cells, which contain no granules in their cytoplasm (Figs. 17, 18, 19). In early infections these latter cells have occasionally outnumbered the myelocytes with which they were associated. The myeloid tissue usually present is quite excessive, frequently forming areas visible to the naked eye. Bacteria have never been found in these lesions.

The *kidneys* are always considerably swollen and of pale, yellowish brown color. Large foci of myelocytes and basophilic, mononuclear cells apparently arising about the blood vessels, are regu-





the effect of bacteria-free filtrates prepared from virulent cultures of *B. aertrycke*.

From a 200 cc. flask of a 24 hour broth culture of *B. aertrycke*, 100 cc. was passed through a VV Berkefeld filter. To test its sterility 40 cc. of this filtrate was immediately inoculated into a fresh flask of sterile broth, and this flask, together with the flask containing the remaining 60 cc. of filtrate, incubated overnight. No growth of bacteria occurred in either of these two flasks. Each of six apparently normal pigeons, whose blood had been found free of natural agglutinins to *B. aertrycke*, was then fed 2 cc. of this sterile *B. aertrycke* filtrate. To test the potency of the organism from which the filtrate was made, 1 cc. of the original broth culture of *B. aertrycke*, then 36 hours old, was fed to each of six other normal pigeons.

As shown in Chart 5, no appreciable changes occurred within twenty-one days in the blood of the six pigeons fed with filtrate, nor did they show any loss of weight or other evidence of disease. When they were killed at the end of this time, no agglutinins for *B. aertrycke* were found in their blood, cultures of liver were sterile in each instance, and no gross or microscopic lesions suggestive of infection with *B. aertrycke* were found in any of their tissues.

All of the six pigeons fed with the original culture of *B. aertrycke* from which the filtrate was made became acutely ill. Four died; two recovered. Autopsies upon the four which died showed all of the characteristic lesions of infection with *B. aertrycke* and this organism was recovered from the organs in each case. The sera of the two birds which recovered agglutinated cultures of *B. aertrycke* in dilutions of 1:320 and 1:2560 respectively one month after infection.

This experiment has since been repeated in a slightly different form. Another sample of filtrate, prepared as outlined above, was fed in amounts of 2 cc. to each of five normal pigeons and also injected intravenously in amounts varying from 0.2 to 1.5 cc. into each of five others. At the same time, as controls, two normal birds were injected intravenously with 1 and 2 cc. of sterile broth, respectively, and each of two others fed with 2 cc. of the same material. The virulence of the culture of *B. aertrycke* from which the filtrate was made was tested by feeding 1 cc. to each of two normal birds.

None of this series of ten pigeons, either fed or intravenously inoculated with *B. aertrycke* filtrate, or the four controls receiving

plugged with necrotic exudate, were caseous, often coalescing areas of consolidation which were sharply demarcated from the surrounding tissue. Microscopically, such areas, within which were myriads of Gram-negative bacilli, were found to be composed of necrotic lung tissue and exudate well encapsulated by a thick zone of mononuclear phagocytes and polymorphonuclear leucocytes. *B. aertrycke* was recovered regularly from such patches of pneumonia. The constant, characteristic distribution of these lesions strongly suggests that they were caused by aspiration of a portion of the culture of bacilli with which the birds were fed. No lesions of any kind have occurred in the lungs of pigeons naturally infected with *B. aertrycke*.

We have never found any significant lesions in the *intestine* of pigeons following natural or experimental infection with *B. aertrycke*. Upon being introduced by mouth, these organisms may be recovered from the stool during the following three or four days in about 50 per cent of the birds. After this time, however, they tend to disappear from the intestine. Intestinal contents and mucosa have been cultured in twenty-six pigeons dying from five to twenty-five days after oral administration of *B. aertrycke*, but in only six instances was the organism recovered. Pure cultures of *B. aertrycke* were grown from the liver of each of these birds and morphologically identical organisms demonstrated in sections of other organs.

The *heart*, *pancreas*, *adrenals*, *thyroid*, *ovaries*, and *testes* have shown no lesions worthy of note. We have examined the *brain* in only four birds infected with *B. aertrycke*, but no lesions were found in these instances.

#### EXPERIMENTS WITH BACTERIA-FREE FILTRATES PREPARED FROM CULTURES OF *B. AERTRYCKE*

Although the oral administration to pigeons of what seemed to be pure cultures of *B. aertrycke* was followed regularly by the disease which we have described, the deduction might not follow that all of the anatomical changes present were caused by this organism. It was clear that the lesions containing bacteria were the direct result of infection with *B. aertrycke*, but the possibility still existed that our cultures were contaminated with a filterable microorganism or contained some filterable agent which was responsible for the changes in the myeloid cells. Accordingly it was decided to test upon pigeons





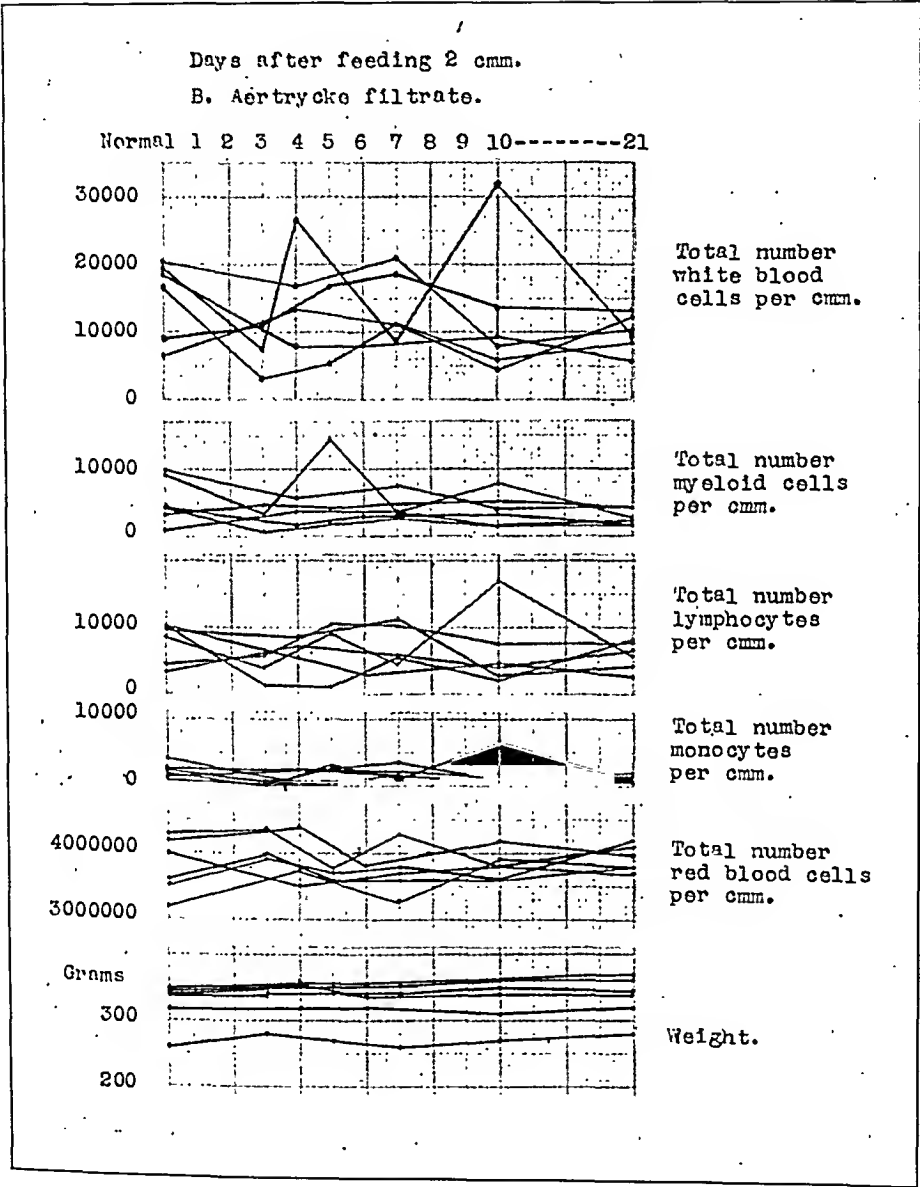


TABLE III

*Summary of Pathological Changes in Sixteen Normal Pigeons used as Controls for Pigeons Infected with B. Aertrycke*

No.	Period of observation	Cultures of liver	Agglutination <i>B. Aertrycke</i>	Pathological changes in tissues
7	10 months	No growth	1:64	Moderate increase in small, round cells about blood vessels of liver and kidney. In a few instances round masses of these cells resembling germinal centers of lymph glands seen
8	10 months	No growth	o	Three small groups of adult polymorphonuclear leucocytes with an occasional myelocyte among them seen adjacent to portal veins
10	10 months	No growth	o	Small round cells (lymphocytes?) slightly increased about vessels of liver
11	10 months	No growth	1:32	Enormous increase of lymphocytes about vessels of liver. Moderate increase of such cells in kidney
12	10 months	No growth	o	No lesions
13	10 months	No growth	o	No lesions
72	1 week	No growth	o	Very slight increase of round cells about vessels of liver
73	1 week	No growth	o	Considerable increase round cells in liver. Small healing necroses in liver and also small nodules consisting of central giant cells and debris surrounded by large, clear monocytic cells and lymphocytes. No tubercle bacilli demonstrable. These lesions do not resemble those of tuberculosis
75	1 week	No growth	o	No lesions
77	1 week	No growth	o	Liver shows nodules as in Pigeon 73. Moderate hyperplasia of bone marrow
79	1 week	No growth	o	Liver shows 1 nodule as in Pigeon 73
81	1 week	No growth	o	Moderate increase of round cells in liver, grouped about which are a few adult polymorphonuclear leucocytes and an occasional myelocyte. Many small basophilic cells seen about vessels and in interstitial tissue of kidney. Moderate hyperplasia of bone marrow
82	1 week	No growth	o	Periportal round cells of liver moderately increased. Also a few polymorphonuclear leucocytes and an occasional myelocyte seen in these areas. No increase of round cells in kidney but 1 small patch of myelocytes seen
84	1 week	No growth	o	No lesions
85	1 week	No growth	o	No lesions
88	1 week	No growth	o	No lesions

sterile broth showed any change in blood picture, loss of weight or other evidence of disease during the ensuing month after administration of these substances. When killed at this time no gross or microscopic changes in any way resembling the lesions occurring in *B. aertrycke* infection were seen. Cultures of the liver made upon blood agar plates were universally sterile, and the blood in each case showed no agglutinins for *B. aertrycke*. Both of the controls, upon which the virulence of the culture of *B. aertrycke* was tested, died within ten days. They showed all of the characteristic symptoms and lesions produced by this organism, which was recovered from the livers of both birds.

Therefore, inasmuch as two different series of pigeons, in all numbering sixteen birds, either fed with or intravenously injected with large, single doses of broth filtrate prepared from virulent cultures of *B. aertrycke* have shown no clinical, anatomical, bacteriological or serological evidence of disease, it seems at least highly probable that all aspects of the disease-picture regularly occurring in pigeons after experimental infection with *B. aertrycke* are due entirely to that organism or its products. However, the possibility that a virus or other substance may be concerned in the alterations of the myeloid tissue is still to be kept in mind until the question has been studied further.

### STUDIES UPON NORMAL PIGEONS

In order to gain some idea of the frequency with which pathological changes occur within the tissues of apparently normal pigeons, and to serve as a general control for our observations upon pigeons infected with *B. aertrycke*, we have studied sixteen birds which gave the appearance of being entirely free of disease. The results are summarized in Table III.

All of the six pigeons, which are stated to have been under observation for ten months, were kept in separate cages but in close proximity to the cages of pigeons infected with *B. aertrycke*. During this time their bloods were examined at frequent intervals but no significant changes were observed; none of them showed any loss of weight or other evidence of disease. The remaining twelve birds observed for one week before being killed for study were well nourished, took their food well, and their blood cells, upon two examinations, showed no pathological changes.

pigment, numerous healing necroses in the liver, and many circumscribed caseous areas in the lungs, each surrounded by a wall of giant cells and connective tissue. These latter lesions, obviously the result of the characteristic lobular pneumonia produced by *B. aertrycke*, varied from microscopic size to as much as 7 mm. in diameter. The smaller ones were identical with the larger healing necroses found in

TABLE IV

*Agglutination Titer of Blood Serum to B. Aertrycke*

No.	Before infection	After infection		
		1 month	5 months	9 months
15	0	..	1:1280	0
16	0	0	1:160	..
20	0	1:2560	0	..
22	0	1:80	0	..
25	0	1:1260	0	..
36	0	0	0	..
38	0	1:320	0	..
42	0	1:320	1:80	..

the liver and also bore close resemblance to the nodular lesions frequently found in the liver of apparently normal pigeons. There was almost no trace left of the myeloid hyperplasia which may have existed during the phase of acute disease in this series of pigeons. In three birds, quite numerous small groups of adult polymorphonuclear leucocytes were seen about the blood vessels in the liver. The bone marrow had returned to its normal appearance in six of the pigeons, but in the two remaining birds gelatinous degeneration of the marrow of the radius was present.

## DISCUSSION

A number of previous workers have already called attention to unusually high leucocytoses varying from 200,000 to 600,000 cells per cmm. which characteristically occur in birds during the course of certain bacterial infections. Among these may be mentioned Smith and Moore,<sup>6</sup> who described a disease of fowls caused by an organism to which they gave the name *B. sanguinarium*, Burckhardt,<sup>7</sup> and Hirschfeld and Jacoby,<sup>8,9</sup> who studied fowls infected with

As seen in the table, cultures of the liver made upon blood agar plates were universally sterile, but the sera of two of the pigeons which had been kept for a long period of time in close proximity to birds infected with *B. aertrycke* were found to agglutinate *B. aertrycke* in dilutions of 1:64 and 1:32 respectively. No agglutinins were present in the bloods of any of the other birds. Attention is called to the frequency with which pathological changes are found in the liver and kidneys of apparently normal pigeons, also to the fact that occasional myelocytes are present in the tissues of such birds. No noteworthy changes of any kind were found in the bone marrow, lungs, spleen, testes, ovaries and intestine.

#### STUDIES UPON PIGEONS SURVIVING EXPERIMENTAL INFECTION WITH *B. AERTRYCKE*

Bacteriological, serological and anatomical studies have been made upon eight pigeons which survived experimental infection with *B. aertrycke* for periods of time varying from five to nine months. All of these pigeons were well nourished and showed no alterations in the peripheral blood or other manifestations of disease when killed for study. Each of these birds had, however, become clinically ill, had lost weight and shown marked increase of the myeloid elements of the blood for a period shortly after oral administration of *B. aertrycke*.

Bacteriological cultures made routinely from the liver on blood agar plates were universally sterile, and cultures of the intestinal contents and mucosa failed to show pathogenic organisms of any kind.

The results of the serological studies are shown in Table IV.

The changes found in the tissues of these pigeons, though of considerable extent, cannot be fully understood until the histogenesis of the lesions produced by *B. aertrycke* has been studied further. Briefly stated, they consisted almost entirely of the healing of the destructive lesions caused by the bacteria. Pigeon 15, killed nine months after infection, showed only one small, encapsulated, caseous area in the lung, the remains of a small patch of pneumonia, no changes of any kind being present in any of the other tissues. The other seven pigeons, killed five months after infection, showed many large phagocytic cells in the liver and spleen loaded with iron

here described, in which no bacteria were found, other widely spread lesions of a totally different character, consisting of accumulations of mononuclear phagocytic cells, containing *B. aertrycke* in abundance, occurred with regularity.

Inasmuch as we have not yet had the opportunity of studying fowl leukemia we can make no definite statement concerning the relationship of this disease to *B. aertrycke* infection in pigeons, though one would be inclined to consider the two conditions as separate entities. Most of our knowledge of fowl leukemia is derived from the studies of Ellermann and Bang,<sup>11</sup> Ellermann,<sup>12, 13, 14, 15</sup> Hirschfeld and Jacoby,<sup>8, 9</sup> and Schmeisser,<sup>16, 17</sup> who, in general, agree that the disease is caused by a filterable microorganism. Ellerman and Bang, who first advanced this theory, did so upon the ground that they were able to transmit the disease to a certain percentage of normal fowls by the injection of bacteria-free filtrates prepared from the organs of a fowl dying of the disease spontaneously acquired. These authors, however, do not appear to have searched very thoroughly for bacteria. No mention is made of cultures made directly from the organs of the birds spontaneously or experimentally infected, nor were the tissues of these birds examined microscopically for bacteria. The only proof offered for the sterility of the filtrate with which the disease was experimentally produced is the statement that it was clear and gave rise to no growth when cultured in several types of media. The amount of filtrate cultured was not mentioned, but it was stated that cultures of the emulsion of tissues from which the infectious filtrate was made gave a rich growth of bacteria. No further study was made of these organisms. Though it is possible that the filtrate with which Ellermann and Bang claimed to have produced leukemia in fowls was actually free of bacteria, one does not feel convinced that such was the case from the account of their experiments. Hirschfeld and Jacoby,<sup>9</sup> who attempted to repeat the experiments of Ellermann and Bang,<sup>11</sup> began their studies with a leukemic fowl obtained directly from the latter workers and were apparently successful in transmitting the disease to other fowls by injections of whole organ emulsion prepared from the diseased bird. However, their stock of fowls proved to be heavily infected with spontaneously acquired tuberculosis. The results of their experiments are not altogether clear, though they studied great numbers of birds in their attempt to separate the effects of these two diseases. It is

avian tuberculosis. So great was the number of myeloid cells in the blood that Smith and Moore considered the disease which they studied to be a form of myeloid leukemia, while Burckhardt expressed the view that myeloid leukemia in fowls was a manifestation of tuberculosis. Hirschfeld and Jacoby oppose Burckhardt's opinion. We can find no blood studies upon pigeons infected with tuberculosis but have ourselves observed a pigeon dying of naturally acquired tuberculous infection whose blood contained 185,000 leucocytes per cmm., of which 92 per cent were mature polymorphonuclears. Cultures of the blood and organs of this bird on blood agar plates and broth were sterile. Though the bone marrow showed marked myeloid hyperplasia, the cells present were mostly adult leucocytes or myelocytes well advanced toward maturity. No myelocytes were seen in the blood and there was no infiltration of other organs with myeloid cells. The mouth, pharynx and bones of the paranasal sinuses showed large caseous lesions, while the liver and spleen contained countless small tubercles. In all of these lesions masses of acid-fast bacilli were present.

None of the observers who have studied these hyperleucocytoses of birds caused by bacteria has reported the presence of an appreciable number of immature white cells in the peripheral blood or the occurrence of infiltrations of such cells in the viscera. We therefore agree with Opie,<sup>10</sup> who recently expressed the view that those investigators reporting the experimental production of diseases closely related to leukemia by means of bacterial infection have failed to justify their claim.

In the case of both natural and experimental infection of pigeons with *B. aertrycke*, however, the situation seems to us to be somewhat different from that of other bacterial infections of birds hitherto reported, though we are by no means prepared to state that this disease is in any way fundamentally related to myeloid leukemia of birds, mammals or human beings. At the present stage of our studies of *B. aertrycke* infection in pigeons we can call attention only to the common occurrence of large numbers of immature myeloid cells in the peripheral blood and the constant appearance of extensive myeloid foci in the liver and kidneys of birds infected with this organism. These changes, to our knowledge, have not been observed to occur in other bacterial infections. We wish to emphasize the fact that, in addition to the hyperplasia and heterotopia of the myeloid tissue



of *B. aertrycke* infection in pigeons is, in itself, a matter of considerable interest. Our observations, though inadequate as absolute proof, would point strongly to the assumption that pigeons frequently harbor minimal numbers of this organism which, under ordinary conditions, cause them no harm. However, under adverse conditions, such as severe malnutrition, it would seem that these bacteria might multiply and frequently become sufficiently pathogenic to produce disease.

That *B. aertrycke* infection is a natural disease of pigeons is shown by observations recently made upon a bird noted to be seriously ill when brought into the laboratory. Immediate examination of the blood showed 2,900,000 erythrocytes and 108,000 leucocytes per cmm., eosinophilic leucocytes 64 per cent, myelocytes 2 per cent, lymphocytes 20 per cent, and monocytes 14 per cent. A culture of the blood made at the same time was positive for *B. aertrycke*, which was identical in all of its characteristics with the strains isolated from spontaneous cases of *B. aertrycke* infection developing in pigeons in the laboratory in New York. On the following day a second blood culture was also positive for *B. aertrycke*. This pigeon was isolated from the other birds and observed for eight days, during which time it remained critically ill, continued to lose weight, and the granulocytes of the blood increased to 83 per cent. After death on the eighth day, all of the lesions characteristic of *B. aertrycke* infection were found in advanced degree and the organism was recovered from the liver, kidney, bone marrow and blood. Intraperitoneal injection of saline emulsion made from the liver, as well as pure cultures of the bacteria, produced both symptoms and lesions in normal pigeons identical with those occurring after experimental infection with other strains of *B. aertrycke*.

We have not yet studied the effect of oral administration of *B. aertrycke* upon animals other than pigeons, but have found these organisms to be highly pathogenic for chickens, mice, guinea pigs and rabbits when injected in fairly large doses. The animals died of septicemia within a few days but did not show myeloid alterations in their organs such as those seen in pigeons infected by the oral route.

Though *B. aertrycke* is generally mentioned in the literature in association with food poisoning in man, it also has been observed to cause disease in animals. The system of nomenclature for the group

worthy of note that Hirschfeld and Jacoby did not succeed in producing leukemia in any of their fowls by the injection of bacteria-free filtrates made from the organs of leukemic birds. Schmeisser,<sup>16, 17</sup> has studied a disease of fowls considered to be myeloid leukemia, which he found to be transmissible to normal birds by injection of organ emulsions made from both spontaneously and experimentally infected birds. He has written a good description of the changes in the blood and other organs but in neither of his papers has he mentioned any bacteriological studies whatever.

Just as this paper was completed the publications of Furth and Stubbs,<sup>33, 34</sup> appeared, confirming once more the filterable nature of the etiological agent in fowl leukemia. While the similarity is striking in certain phases of the hemopoietic response, it would seem that entirely different causative agents were at work in fowl leukemia and *B. aertrycke* infection in pigeons. Though all of these investigators who have studied fowl leukemia have described lesions apparently identical with the changes in the myeloid tissue occurring in pigeons infected with *B. aertrycke*, none has reported the presence of the widely spread collections of monocytic cells frequently grouped about bacteria which we have regularly observed. It seems unlikely that such lesions would be overlooked. Furthermore, whereas the incubation period of fowl leukemia is stated to be approximately two months, our pigeons infected with large doses of *B. aertrycke* became ill within two days, and small doses of these bacteria produced no symptoms of disease at all. In relation to this latter point may be mentioned the work of Winternitz and Schmeisser,<sup>18</sup> who studied a series of fowls experimentally infected with *B. sanguinarium*. As previously found by Smith and Moore, their birds developed high leucocytoses but showed no extramedullary accumulations of myelocytes. A single bird, however, developed changes in the blood characteristic of myeloid leukemia several weeks after injection of the bacteria and was found to show extensive infiltration of the liver and kidneys with myelocytes. No bacteria of any kind were recovered from this bird and these investigators were inclined to conclude that the case was one of true fowl leukemia. They suggested the possibility that leukemia may be produced in the fowl by graded doses of *B. sanguinarium*, but did not pursue the matter further.

Aside from its possible relationship to fowl leukemia, the question

bladder, purulent endometritis, infrequent pneumonia and cloudy swelling of kidneys. No mention was made of the bone marrow.

Though the literature contains several references,<sup>29, 30, 31</sup> other than instances of food poisoning, to human cases of fatal infection with *B. aertrycke*, none of them has resembled the disease caused by this organism which we have studied in pigeons. From the accounts of such cases it seems that *B. aertrycke* was recovered only from the blood, and was never proved to be the cause of any of the lesions present.

#### SUMMARY AND CONCLUSIONS

The apparently spontaneous development of a fatal disease in undernourished pigeons is reported which is characterized by anemia, marked myeloid hyperplasia of the bone marrow, striking increase of the myeloid elements of the blood, and extensive infiltration of the liver and kidneys with myeloid tissue. In addition to these myeloid changes, large, nodular, often necrotic masses of mononuclear phagocytic cells are frequently found scattered throughout the liver, spleen, kidneys and bone marrow.

A small, Gram-negative bacillus, regularly recovered in pure culture from the blood, liver, kidney, spleen, and bone marrow of these cases, has been identified as *B. aertrycke*. In sections, the bacteria are found to be present in the foci of mononuclear cells, but do not occur within the collections of myelocytes.

Disease has been produced experimentally in normal pigeons by the intraperitoneal injection of liver emulsion made from naturally infected birds, intraperitoneal injection of *B. aertrycke* derived from the same source, and also by oral administration of single large doses of broth cultures of this organism.

Bacteria-free filtrates of broth cultures of *B. aertrycke* have had no demonstrable effect upon normal pigeons when injected or administered orally in single large doses.

Attention is called to the frequency with which pathological changes occur in the tissues of apparently normal birds.

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of bacteria related to *B. aertrycke* is so inaccurate, however, that relatively few of the investigators who have reported such observations appear to have been dealing with the same organism. Otte<sup>19</sup> has reviewed the many studies upon natural and experimental infection of birds with bacteria of this type, and Beck and Meyer<sup>20</sup> have studied a disease of pigeons, widespread in Germany, which was caused by a bacillus considered to be *B. aertrycke*. No blood studies were made by any of these observers. Infiltrations with mononuclear cells, similar to those we have observed in pigeons, were of common occurrence but no changes in the myeloid cells were reported.

Beaudette and Edwards<sup>21</sup> have reported an epidemic in canaries caused by this organism, Beaudette<sup>22</sup> an epidemic in young pigeons, and Doyle<sup>23</sup> has described an epidemic among young chicks. Meyer and Matsumura<sup>24</sup> state that a considerable percentage of wild rats are carriers of *B. aertrycke*. Petrie and O'Brien<sup>25</sup> described an epidemic in guinea pigs which they thought was due to a filterable microorganism, but regularly cultured *B. aertrycke* from the dead animals. This organism was highly pathogenic for normal guinea pigs when injected subcutaneously, but of low virulence when administered by mouth. Topley and Ayrton,<sup>26</sup> working with white mice, found the greatest variation in the behavior of different strains of *B. aertrycke*. Some strains were excreted regularly from the intestine after oral administration, while others appeared only transiently, in some instances fatal infection taking place without the organisms ever appearing in the feces. These workers also showed that *B. aertrycke* frequently remained in the tissues of mice without causing disease. Topley,<sup>27</sup> at a later date, demonstrated *B. aertrycke* of unaltered virulence in the spleens of mice actively immunized to lethal doses of this organism. Edington<sup>28</sup> has recently made a very thorough study of another guinea pig epidemic caused by *B. aertrycke* in which this organism was recovered regularly from the blood, intestine, gall-bladder, liver, spleen and urine. Blood studies showed a leucopenia varying from 2,000 to 4,000 cells per cmm. with no striking change in the differential count. The lesions found were empyema of the gall-bladder, necrosis in the spleen and liver, hyperemia of the intestine and occasional small ulcerations in the lymphoid follicles of the mucosa, accumulations of phagocytes with necroses in the mesenteric lymph nodes, catarrhal inflammation of urinary

## DESCRIPTION OF PLATES

## PLATE 67

Cells stained supravitaly with neutral red and Janus green from the peripheral circulation of pigeons in this experimental series.

- FIG. 1. Young adult eosinophilic leucocyte showing mitochondria and a mixture of spheres and rods making up the specific granules. These are the first changes to appear in the circulating granulocytes of the pigeon under stress. Pigeon 70, April 16, 1929, size  $9 \times 12$  microns.
- FIG. 2. Young adult eosinophilic leucocyte with mitochondria. Pigeon 65, peripheral blood, April 3, 1929, size  $9 \times 9\frac{1}{2}$  microns.
- FIG. 3. Atypical micro-eosinophil with swollen rods not infrequently found in the circulation as an indication of bone marrow stimulation. Pigeon 65, April 3, 1929, size  $8 \times 9$  microns.
- FIG. 4. Adult eosinophilic leucocyte showing the incomplete transition of spherical granules to rods and indicating a premature delivery of this cell to the circulation. Pigeon 65, April 3, 1929, size  $10 \times 15$  microns.
- FIG. 5. Eosinophilic myelocyte "B" with round nucleus, mitochondria and a moderate number of specific, spherical granules. Pigeon 65, April 3, 1919, size  $10 \times 10$  microns. See erythrocyte for relative size.
- FIG. 6. Eosinophilic myelocyte "A" with many mitochondria and few specific granules. Size  $9 \times 10$  microns. Note the different stages of maturation found within the eosinophilic group on the same day from Pigeon 65, April 3, 1929.
- FIG. 7. Eosinophilic myelocyte "C," showing mitochondria and spherical granules arranged about the centrosphere, a not infrequent arrangement in the myelocytic stage. The slightly indented achromatic nucleus contains one nucleolus. Pigeon 50, March 22, 1929, size  $10\frac{1}{2} \times 12$  microns.
- FIG. 8. Myelocyte "B" contains approximately one-half the final concentration of specific granules found in the fully mature eosinophil and many mitochondria. The area about the centrosome is clear of granules. The nucleus shows very little chromatin structure at this stage of development. Pigeon 50, March 22, 1929, size  $15 \times 15$  microns.
- FIG. 9. This myelocyte "A" has relatively few specific granules, several vacuoles, and many mitochondria. The nucleus contains two nucleoli and very little chromatin. Size  $12 \times 13\frac{1}{2}$  microns. Cells 7, 8 and 9 show the various stages of myelocytic maturation found on the same day in the blood of Pigeon 50, March 22, 1929.
- FIG. 10. Myelocyte "B." Pigeon 65, April 4, 1929, size  $9 \times 12$  microns.
- FIG. 11. Myelocyte "A." Pigeon 65, April 4, 1929, size  $10 \times 14$  microns.
- FIG. 12. Monocyte from the peripheral blood of Pigeon 50, March 21, 1929. Note the slightly different shade of neutral red reaction in the vacuoles of this cell as contrasted with the granules in the myelocytes of Figs. 7, 8 and 9 from the same bird. See accompanying erythrocyte for relative size.
- FIG. 13. Monocyte from Pigeon 65, April 4, 1929. Contrast this cell with the myelocytes of Figs. 10 and 11 taken from the same bird on the same date. Size  $9 \times 10\frac{1}{2}$  microns.
- FIG. 14. Small lymphocyte with many mitochondria and a few scattered neutral red vacuoles. Pigeon 67, April 4, 1929, size  $9 \times 9$  microns. See accompanying erythrocyte for relative size.

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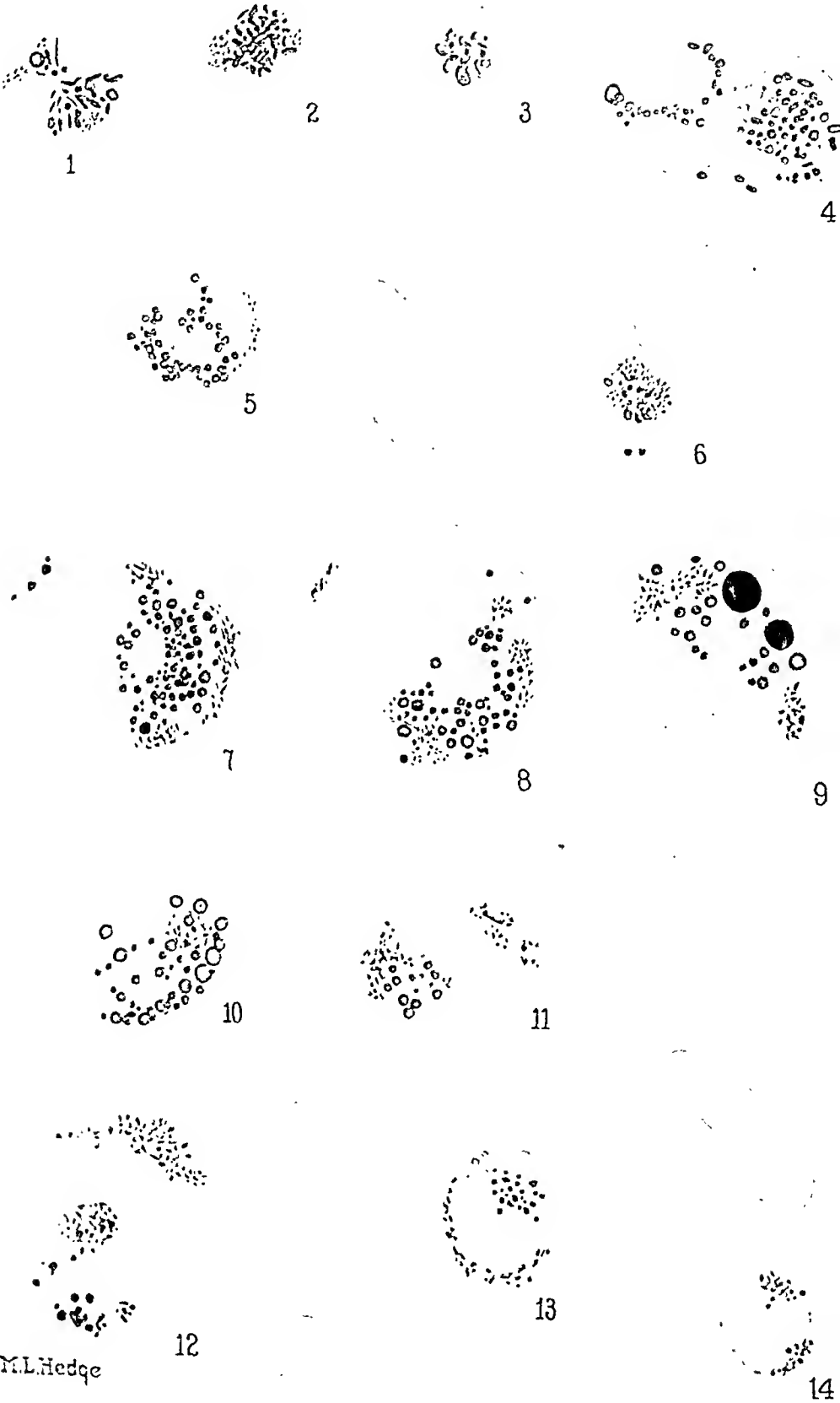
## PLATE 68

FIG. 15. Liver of Pigeon R714, naturally infected with *B. aertrycke*, showing unusually large collection of monocytic cells, in which there is a necrotic area containing Gram-negative bacilli. Hematoxylin-eosin stain.  $\times 62$ .

FIG. 16. Smaller collection of monocytic cells in liver of Pigeon 24, experimentally infected with *B. aertrycke*. There are bacteria and a minute area of necrosis in the center about which is heavy infiltration with adult polymorphonuclear leucocytes. Hematoxylin-eosin stain.  $\times 300$ .

FIG. 17. Low power picture of liver of Pigeon 14, experimentally infected with *B. aertrycke*, showing general distribution of infiltrations of myeloid cells.  $\times 62$ .

FIG. 18. Small branch of portal vein with surrounding zone of myelocytes. Experimental infection *B. aertrycke*, Pigeon 14. Hematoxylin-eosin stain.  $\times 300$ .



M.L.Hedge



PLATE 69

FIG. 19. Myelocytes surrounding portal vein. A small portion of the vessel filled with erythrocytes may be seen in the upper left-hand corner. Natural infection with *B. aertrycke*, Pigeon 14. Hematoxylin-eosin stain.  $\times 1300$ .

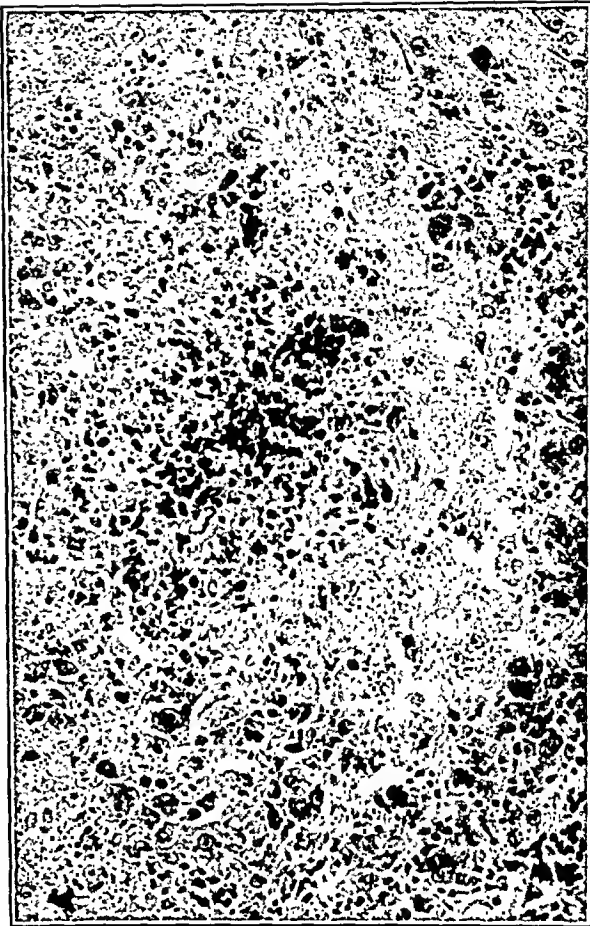
FIG. 20. Infiltration of kidney with myelocytes. Experimental infection with *B. aertrycke*, Pigeon 14. Hematoxylin-eosin stain.  $\times 158$ .

FIG. 21. Bone marrow from central portion of shaft of radius of Pigeon 30 dying of experimental *B. aertrycke* infection. The partially collapsed capillaries are marked out by the characteristic nuclei of erythrocytes. All of the remaining cells are myelocytes of varying degrees of maturity. The more mature forms appear darker than the others due to their greater number of granules. The degree of myeloid hyperplasia may be appreciated by bearing in mind the fact that the capillary network of this portion of the marrow of normal pigeons contains only fat among its meshes. Hematoxylin-eosin stain.  $\times 300$ .

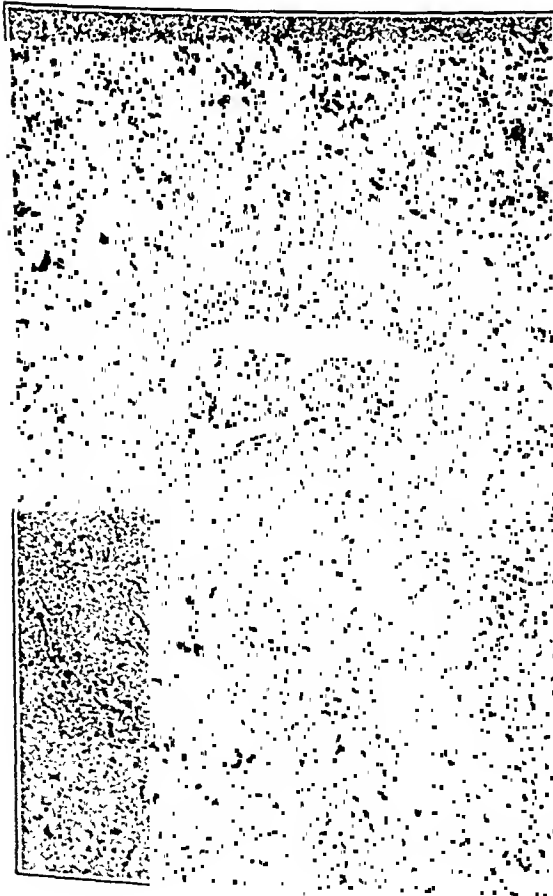
FIG. 22. Oil-immersion picture from same marrow as Fig. 7, illustrating the pale staining, myeloid forms with only a few granules in their cytoplasm. A few mature myelocytes with their full quota of granules are also seen. Hematoxylin-eosin stain.  $\times 1300$ .



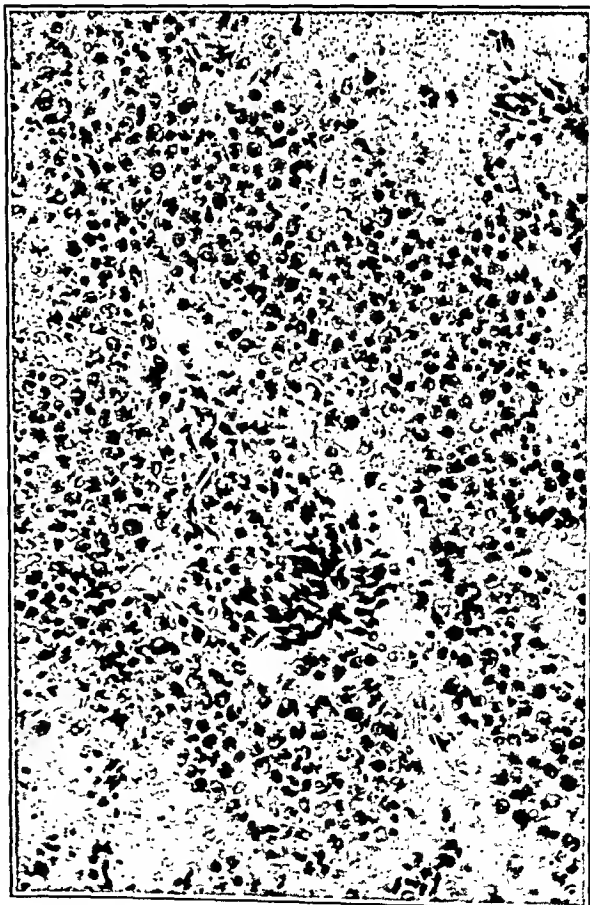
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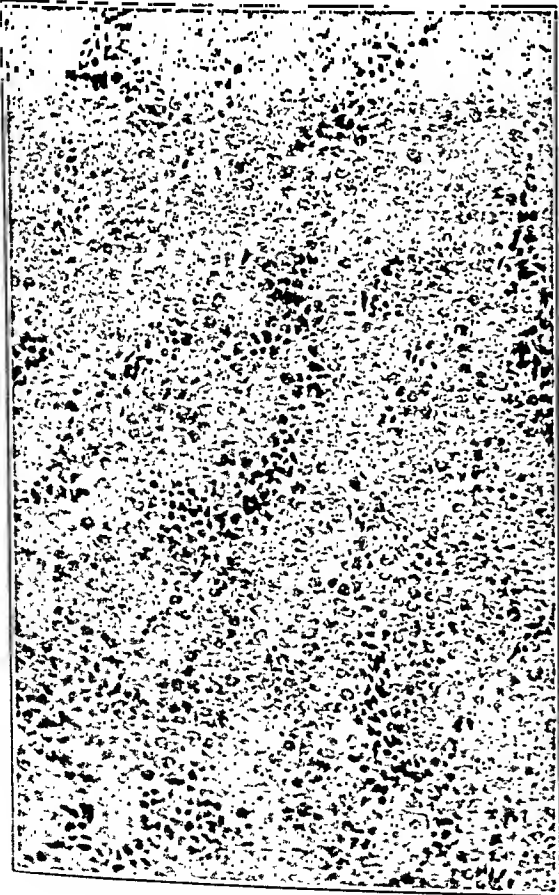




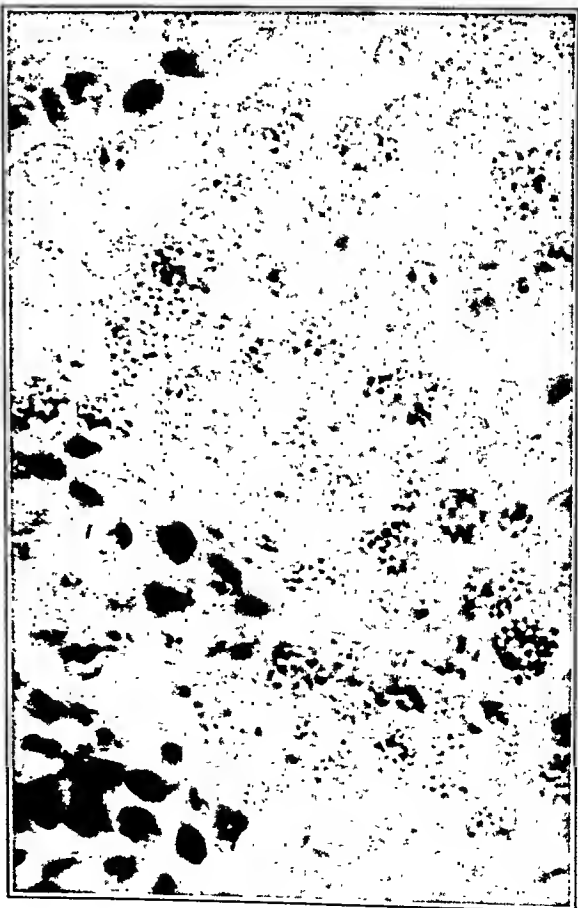
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## MATERIAL AND METHODS

In all, two hundred and seventeen carpometacarpal joints of bovine embryos, calves and cattle were examined. The joints of eight embryos ranging from 5.2 cm. to 70 cm. in length were subjected to macroscopic and microscopic examination. Thirty joint specimens obtained from slaughtered calves were examined grossly. Histological studies of selected ones were made. The joints of one hundred steers and heifers between 1 and 5 years of age and of fifty older milch cows were examined macroscopically. Finally, thirty-seven specimens which showed the minimal to maximal sized lesions in the two types of animals, young steers and heifers (beef cattle) and the older milch cows, were selected. All of this latter group of specimens were used for macroscopic and microscopic study. From them, the gross and microscopic illustrations were made.

The specimens were all fixed in 10 per cent formaldehyde solution. Large or complete transverse or anteroposterior blocks of cartilage and subchondral bone were taken. These blocks were decalcified in a 5 per cent nitric acid solution and embedded in celloidin. Sections were stained with hematoxylin and eosin. A few of the embryo specimens were embedded in paraffin, as were the synovial membrane specimens. Serial sections were made from a few of the celloidin blocks, employing a modification of the technique described for use in frozen sections.<sup>2</sup>

DEVELOPMENT OF ARTICULAR CARTILAGE IN THE  
CARPOMETACARPAL JOINT

*Macroscopic Description:* The carpometacarpal joints of a 5.2 cm. embryo were too small to warrant gross description. In a 15 cm. embryo, the metacarpal articular cartilage measured 3.5 mm. in width by 2 mm. in depth. In the larger embryos, the articular cartilage had increased sufficiently in size, so that in the oldest (70 cm.) embryo, it measured 30 mm. in width by 3 to 3.5 mm. in thickness. Small blood vessels just visible to the unaided eye were seen in all the larger specimens of articular cartilage.

The macroscopic examination of the carpometacarpal joints of thirty-five calves which were approximately 6 to 12 weeks of age failed to reveal any evidence of articular cartilage degeneration. The surface of the cartilage was smooth and glistening (Fig. 1). Occa-

## A SYSTEMATIC STUDY OF THE DEGENERATION OF ARTICULAR CARTILAGE IN BOVINE JOINTS \*

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In a previous study concerning the cytology and nitrogen content of normal synovial fluid of cattle,<sup>1</sup> constant differences were noted in the fluid removed from the carpometacarpal and astragalotibial joints. Synovial fluid obtained from the carpometacarpal joints was more viscid, contained more nucleated cells per cubic millimeter and showed a higher total protein content than did synovial fluid obtained from the astragalotibial joints of the same animal. At that time, occasional macroscopic and microscopic examinations of these joints revealed constant areas of degeneration in the medial articular cartilages of the carpometacarpal articulations. The astragalotibial joints which were examined<sup>1</sup> did not reveal similar lesions. A brief comment concerning these areas of degeneration in cartilage was made. The constant differences in the synovial fluid obtained from these carpometacarpal joints as compared to the synovial fluid aspirated from the astragalotibial articulations was explained by their presence.

The present investigation was undertaken with the purpose of studying these degenerative changes in the articular cartilage from their beginning through all the stages of development and if possible of assigning the causes for their occurrence. It was also hoped that a detailed study of the initial lesions might enable us to understand better the earliest pathological changes which occur in diseases of the articular cartilage in man.

A study was made of the carpometacarpal joints in a series of embryos in order to determine whether or not constant differences in development or peculiarities in vascular supply played any part in the production of these lesions.

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carpal bones. The deepest zone of cartilage consisted of columns of flattened cells; in the upper two-thirds the cells were rapidly dividing, whereas in the lower one-third the cells were undergoing degeneration, prior to bone formation.

Embryos of 59 and 70 cm. length showed articular cartilages which had markedly increased in width. Evidences of rapid growth were still present in the form of fairly numerous mitotic figures and incomplete ossification of the subchondral bone trabeculae. Blood vessels of large size were seen entering from the margins of the cartilage into its middle zone where they immediately branched into many smaller divisions. One noted in the study of these serial sections that many of the blood vessels just above the line of ossification were obliterated. No blood vessels were seen to enter from the subchondral bone.

Sections obtained from growing calves showed a thinner cartilage which was mature from the standpoint of both cells and matrix (see Fig. 3). Fewer blood vessels were present. The three merging layers of cells were less distinct. The cartilage cells were nearly always in pairs or groups of cells and were arranged in an orderly manner. No mitotic figures were seen. A more abundant matrix was present and the subchondral bone was more completely calcified. The line of ossification in all preceding specimens was even and (in brief) consisted of buds of capillaries growing into the degenerating columns of cartilage cells. Oval, elongated and polyhedral cells (osteoblasts) accompanied these blood vessels and a pink-staining homogeneous osteoid matrix was laid down about them. This matrix was built up into regular, evenly spaced bone trabeculae. In the specimens obtained from young calves, the subchondral bone had encroached sufficiently upon the vascular zone of cartilage, so that occasional blood vessels were being surrounded by bone above the line of ossification (Fig. 3). This overtaking of the vascular zone of articular cartilage by subchondral bone suggests the process by which adult cartilage becomes avascular. In all the embryo specimens, the transition of cartilage into bone was by direct replacement from below. In calves, a calcified layer of cartilage had begun to form (Fig. 3). In older animals the deepest layer of cartilage was calcified, as indicated by its staining reaction (Fig. 4).

sional small blood vessels could be seen in the depth of the cartilage by superficial inspection. After shaving off the surface cartilage, a rich blood vessel and capillary network was seen readily with the aid of a hand lens. Vertical transverse cross-sections showed the articular cartilage to average approximately 1 mm. in thickness. The metacarpal articular cartilage in animals older than those already described was reduced to a fraction of a millimeter in thickness. It should be emphasized that in the metacarpal bones of the cow there is no epiphysis. Thus, the thick articular cartilage of the embryo gradually becomes transformed into a thin adult articular cartilage.

*Microscopic Description:* A longitudinal section through the fore leg of the smallest (5.2 cm.) embryo showed the bulk of tissue to consist of cell-poor embryonic connective tissue. In the area where the carpometacarpal articulation later develops, there had been an accumulation of mesenchymal cells. This compact group of cells had differentiated, in its central portion, into avascular embryonal cartilage, while the peripheral zone of cells appeared to consist wholly of fibroblasts. No cleavage into a joint space had occurred in the region of the carpometacarpal joint, although a well formed articular cavity had developed at the radiohumeral joint. With the exception of the humerus, where some bony matrix had been laid down, the bone anlage consisted wholly of avascular embryonal cartilage. A few blood vessels, however, were noted in the surrounding cellular connective tissue (periosteum).

Longitudinal sections through the carpometacarpal joints of embryos 15 to 59 cm. in length revealed well developed bones, articular cartilages and joint cavities. In these specimens the cartilage had differentiated into three zones: (1) a superficial or perichondral, (2) a middle or vascular, and (3) a deep or proliferating zone (see Fig. 2). These three zones merged one into another without sharp lines of distinction. Numerous mitotic figures were found in all three zones. The surface zone of cartilage consisted of three to five layers of cells, the most superficial layer of which had the morphology of fibroblasts. The middle zone was composed of irregularly shaped and placed embryonal cartilage cells. Several medium sized blood vessels were present in this zone. These blood vessels, which usually consisted of artery and accompanying vein, could be traced in all sections except two to the larger vessels in the perichondrium and periosteum at the margins of the joints and the line of fusion between the meta-



cartilage was a pale yellow in color. Various sized lesions in old milch cows are illustrated in Figs. 12, 13 and 14. The bases of several of the larger defects appeared on macroscopic examination to be covered by organizing fibrin. Histological study, however, failed, with one exception, to show any fibrinous exudate.

In occasional joints, at the site of the future degenerative lesions, minute, hard yellowish elevations were found. These nodules did not usually project more than 1 mm. above the cartilage surface and were seldom more than 2 mm. in diameter.

Macroscopically there was little evidence of synovial membrane pathology. No villous fringes were seen and for the most part the synovial membrane was smooth and glistening.

*Microscopic Examination:* A systematic study of sections from twenty-two joints was made. These sections showed all stages of development of the articular cartilage lesions.

The normal portions of the adult articular cartilage showed a thin layer of uniform hyaline cartilage, which was 0.7 to 0.9 mm. in thickness. The articular surface was smooth. At the surface the paired cartilage cells, which were usually two to four layers deep, were so arranged that their long axis was transverse to the vertical axis of the bone. Beneath the surface layer, the cells were grouped within lacunar spaces in clusters of from two to fourteen cells. Many of the cells were in rows and columns. The cartilage matrix appeared homogeneous, purplish in staining reaction and showed no evidence of fibrillation. The matrix at the surface and just above the calcified layer stained slightly more intensely than in the middle zone. Just above the subchondral bone was a layer of calcified cartilage, the upper surface of which was smooth, the lower border forming an irregular line of union with the bone trabeculae below. This zone of calcification was about one-third the thickness of the entire articular cartilage. Blood vessels from the intertrabecular spaces very frequently were found within this zone. The subchondral bone consisted of thick trabeculae which enclosed small spaces containing fat, numerous capillaries and small blood vessels. Normal adult metacarpal articular cartilage and subchondral bone is illustrated in Fig. 4.

The earliest constant histological change noted was a thinning of the layer of calcified cartilage. The affinity of this layer for basic stain was decreased. On the surface of the articular cartilage one

## DEGENERATIVE LESIONS OF ARTICULAR CARTILAGE

*Macroscopic Examination:* In order that the stages of development of these degenerative lesions of cartilage might be more briefly and clearly described, the specimens have been grouped into four classes for both gross and microscopic study: (1) early lesions as illustrated in gross photographs, Figs. 5, 6, 7 and 8; (2) medium sized lesions as in Fig. 9; (3) large lesions of older beef cattle as in Figs. 10 and 11, and finally, (4) defects seen in old milch cows as illustrated in Figs. 12, 13 and 14.

Gross examination of the carpometacarpal articulations of six steers and heifers (beef cattle), which because of the absence of any permanent teeth were assumed to be under 2 years of age, revealed the earliest lesions. These changes are illustrated in Figs. 5, 6, 7 and 8. They consisted of slightly depressed, roughened areas of cartilage, or small areas in which cartilage was completely absent (Fig. 8). These small erosions always occurred in the concave surface of the medial articular cartilage.

Larger and deeper areas of degeneration in articular cartilage were found in the other carpometacarpal joints collected. Fig. 9 illustrates a degenerative lesion of average size and depth for young beef cattle. The area of degeneration in this joint measured 10 by 11 mm. It was sharply outlined by an irregular margin of overhanging cartilage. The base of the defect extended into subchondral bone 1.5 mm. in the deepest portion.

Figs. 10 and 11 illustrate the largest degenerative lesions observed in beef cattle. These lesions measured 20 by 17 by 2.5 mm. and 25 by 7 by 5 mm. respectively. In both instances they had extended deeply into the subchondral bone. The similarity of the size, shape and location of the lesions in the carpal cartilage as compared to the defects in the metacarpal cartilage is well illustrated in Fig. 11. It should be emphasized that these degenerative changes always occurred on the medial half of the joint cartilage and were present in both the carpal and metacarpal cartilages. Usually one defect was the mirror image of the opposing degenerative area and striking similarities between right and left side were noted (see Fig. 10).

In old milch cows, the degenerative lesions, while located in the same areas, presented slightly different appearances. The margins were sharper and less irregular in outline. The surrounding articular

one may state that complete degeneration of articular cartilage and repair by granulation tissue does not necessarily precede vascularization of cartilage. The disappearance of the layer of calcified cartilage appeared to be of fundamental importance.

In the medium sized lesions, as illustrated grossly in Fig. 9, the articular cartilage had completely disappeared in the sharply demarcated area of degeneration. Subchondral bone had disappeared for a depth of from three to five times the thickness of articular cartilage. At the margins of the defects there was an abrupt change from normal hyaline cartilage into fibrocartilage and fibrous tissue. This change was first apparent on the articular surface and only later in the depth of the cartilage. The bases of the defects were lined by fibrous tissue in which fairly numerous small blood vessels were seen. The underlying bone trabeculae showed evidence of atrophy and rearrangement, but no evidence of osteoclasts. The marrow spaces were filled largely with fat but showed no fibrous tissue proliferation (Fig. 19).

The largest lesions studied gave little additional information. More bone trabeculae had been resorbed so that the subchondral bone defects were six times the thickness of articular cartilage. The margins were sharp and sometimes overhanging (Fig. 20). In one instance, definite crushing of the bone trabeculae beneath the articular cartilage was observed. In occasional sections, the connective tissue lining the areas of cartilage degeneration had become very much thickened, extremely vascular, and numerous fat cells had replaced the fibrous tissue (Fig. 21).

In the old cows there was more hyalinization of the connective tissue which lined the depressed lesions. The adjacent cartilage matrix was often more intensely stained with basic dye (Fig. 22).

No important pathology was found in the associated synovial membranes. There were numerous branching small blood vessels in the subsynovial tissue, many of which showed thickened hyalinized walls. There was also a varying degree of chronic inflammatory cell infiltration in parts of the synovial membrane and subsynovial tissues. This feature, however, was never very marked. No pannus formation or synovial villi were observed in any of the joints examined.

frequently observed small light staining elevations at the margins of the beginning depressions. Most of these elevations were partially covered by fibroblasts and were composed of lightly staining matrix which enclosed scattered and distorted groups of cartilage cells. In many instances, the surface cells of these elevations closely simulated the connective tissue (perichondrium) seen at the margins of the articular cartilage (Fig. 15). Associated with the above changes, one frequently observed varying degrees of fibrillation of the cartilage matrix. In its earliest stages this was shown by a basic stain-streaking of the matrix between columns of cartilage cells. The streaking and later fibrillation of the hyaline matrix was almost always in the vertical axis (see Fig. 16). Varying degrees of distortion of the rows and columns of cartilage cells were usually associated with the fibrillation. In a few instances actual vertical splitting of the matrix had occurred. Examination of blood vessels in the subchondral bone beneath these early lesions showed no constant changes. In some instances, they were dilated and engorged; in other instances they appeared deficient in number. As a result of the thinning of the calcified cartilage layer, the subchondral blood vessels were nearer to the articular cartilage proper. It should be emphasized that we never found important inflammatory cell infiltration or necrotic foci in the subchondral bone or marrow spaces.

The small, hard, yellowish elevations noted on the articular surface, when examined histologically, were found to be enlarged overgrowths of articular cartilage such as have already been described (see Fig. 16). Some of these localized outgrowths had as a result of pressure spread out to form overlapping "mushroom-like" margins. The cartilage cells in these elevations were distorted, the matrix was fibrillated and vertical clefts had formed. In some of these protuberances lime salt deposition was observed.

In slightly more advanced lesions, shallow depressions and definite thinning of the articular cartilage had occurred. The calcified zone was markedly thinned, distorted and absent in many places. The surface of these altered areas of cartilage was covered by several layers of cells which could not be distinguished from fibroblasts (see Fig. 17). In more advanced lesions, the calcified layer of cartilage had completely disappeared. All of the remaining cartilage appeared as fibrocartilage and had been invaded by blood vessels from the intertrabecular spaces of the subchondral bone (see Fig. 18). Thus,

lesions were self-limited and not accompanied by any important pathological changes in other portions of the involved joints.

Possible etiological factors concerned with the degenerative lesions of the carpometacarpal articulations have been considered. The gross and microscopic examination showed that they were of a degenerative nature. No lesions were ever found in the carpometacarpal joints of calves slaughtered at the age customarily used for veal. The earliest lesions were found in young beef cattle (under 2 years of age), and the more extensive lesions occurred in some of the older animals. It is important to emphasize that the lesions did not necessarily progress once they had developed. Having reached their maximum size, they remained as depressed areas with little or no attempt to repair.

The degenerative changes in articular cartilage appeared to be unrelated to vascular changes as factors of causation. From the study of embryo and calf specimens it was observed that the blood supply to both the medial and lateral articular cartilage disappeared at the same time, yet the areas of degeneration occurred only on the medial cartilage. These blood vessels had all disappeared in the beef cattle before the age of 2 years. Examination of the blood vessels in the subchondral bone beneath the developing defects in cartilage did not reveal any abnormalities which were constant or of histological importance. The arterioles in the fibrous tissue lining the areas of degeneration (once formed) were often thick-walled and hyalinized. This factor was thought to be secondary to the alterations already present rather than to be in any way responsible for them.

Bacterial infection may be dismissed as a causative factor because of the constant occurrence of this lesion in all animals, the constant involvement of one localized area of the articulation, and because there is no histological or cytological evidence of infection to be found in the synovial membranes, articular cartilage, subchondral bone, or synovial fluid.

Gout as an etiological factor was ruled out since deposits of sodium urate crystals never were found upon articular surfaces or in the articular cartilage of either early or late joint lesions.

The constant occurrence of the localized articular cartilage degeneration in young western beef cattle and older milch cows \* ap-

\* These cattle were obtained largely from the New England states.

## COMMENT

Relatively few studies of the pathology of spontaneous joint diseases of animals have been reported. Such observations have been recorded for the most part in publications which do not readily come to the attention of those interested in the study of human arthritis. The value of an intensive study of arthritis in domestic animals is well illustrated by the work of Hare<sup>3</sup> who, from a careful and extensive pathological study of rheumatoid arthritis in horses (the human proliferative type of Nichols and Richardson<sup>4</sup>), was able to describe in detail the changes in all portions of the involved joints. While areas of degeneration on opposing cartilage surfaces were observed by Hare, they were always associated with other important joint changes which were thought by him to follow inflammatory processes in the vascular connective tissue of the joints and tendons.

Chronic arthritis specifically involving the carpal articulation of horses has been described by Cherry<sup>5</sup> and Krüger.<sup>6</sup> Although Krüger mentioned areas of degeneration of cartilage on opposing articular surfaces, there were other associated articular changes. These changes were lipping, osteophyte formation, proliferation and inflammation of the synovial membrane.

In papers dealing with the disease process termed spavin of the tarsometatarsal articulation of horses and cattle, one finds an occasional brief description of pathological changes comparable to those that are the subject of this paper. However, the process appears to have been entirely different in that various workers<sup>7, 8, 9</sup> have directed attention to the inflammatory nature of the disease and the tendency to ankylosis of the involved joints.

Lesions such as are described in the present study are not mentioned by Huttyra and Marek<sup>10</sup> who, in discussing articular rheumatism of domestic animals, stated that it was most frequently seen in cattle. In cattle this disease affected delicate milkers most commonly, less often oxen, and was scarcely ever encountered in grazing cattle. It was assumed that the disease described (articular rheumatism) was due largely to bacterial infection.

The present investigation deals with progressive lesions of the articular cartilage of cattle which occur on the opposing articular surfaces of the medial side of the metacarpal and carpal bones. These

the seat of marked arthritic changes. From the mechanical standpoint, the carpometacarpal joint of the horse is more nearly perfect than is the corresponding joint of the cow. In the case of the former animal there are more articulating concavities and convexities which would aid in preventing anteroposterior or lateral slipping and the posterior portion of the joint is strengthened by two very strong articular ligaments. The metacarpal articulating surface of the cow is comprised of two large flat surfaces which are divided by a single narrow anteroposterior ridge situated just lateral to the midline. Only one articular ligament connects the carpal and metacarpal bones in cattle. In the cow one finds a considerable degree of genu valgum of the fore leg. Because of this factor, it would appear that the greatest weight is borne on the medial aspect of the carpometacarpal joint. Repeated traumatic injury applied to the articular surfaces of a weakly constructed joint might well explain the lesion in question, particularly since such lesions occur regularly over the area of least bony support. A probable source of repeated trauma is found in the manner in which a cow uses the front knees (carpometacarpal articulations) in the process of lying down and getting onto her feet from the recumbent position. In lying down, the cow drops the weight of the fore quarters upon one sharply flexed carpometacarpal joint and then upon the other. In rising, the front legs are folded under the thorax and the weight of the fore quarters of the animal is carried on the front knees until the hind quarters are upright, when with great exertion, the fore quarters are lifted from one front knee at a time. This series of movements is entirely different from those of the horse where the fore legs are extended forward and the weight of the fore quarters lifted onto the fully extended fore legs with the aid of forceful pushing by the rear extremities. If the above observations are indicative of the stresses applied to the carpal and metacarpal bones of the cow, then these articular cartilage lesions may well be considered as traumatic in origin.

It has been previously stated that the degeneration involved similarly the two opposing cartilage surfaces. This was true in both the early and the more extensive lesions. While this fact is apparently in agreement with a theory of traumatic origin, it does not explain why the two opposing depressed areas extended deeper, once formed. This phenomenon may be explained as follows: Once the lesions are formed, the weight is carried on the surrounding intact articular sur-

pears to rule out differences of activity and habitat as being of importance in causation.

The study of embryo joints failed to reveal anything unusual in the development of the carpometacarpal joints. It was learned from these specimens, however, that the articular cartilage rapidly decreased in thickness in the early months of life. When large transverse sections of the metacarpal articular cartilage and subchondral bone of embryos, calves and cattle were mounted in series on large glass plates and studied with a hand lens, interesting structural changes in the subchondral bone were noted. In the embryos and calves, the subchondral bone trabeculae were evenly distributed beneath the articular cartilage (Fig. 23). In the older animals one noted a rearrangement of the trabeculae and an increase in the thickness of the bone cortex. There was a marked difference in the manner in which the compact cortical bone expanded into the subchondral bone trabeculae of the medial and lateral sides (Fig. 24). The lateral cortex of the metacarpal bone flared sharply from the vertical axis, so that the vertical subchondral bone trabeculae supported adequately the entire lateral articulating surface, whereas the vertical subchondral bone trabeculae branching from the mesial cortex supported only the medial one-half of the medial articular surface. The central area of the entire articular surface was well supported by the dense bone which resulted from the fusion of two metacarpal bones. This rearrangement of bone trabeculae resulted in an area of rarefied subchondral bone which was directly beneath the site of these cartilage defects. These areas of rarefied subchondral bone were continuous with the marrow spaces of the bone metaphysis. The fact that the articular cartilage defects occurred directly over these areas of rarefied bone suggests that they are potential weak spots and therefore of great etiological importance. Such a deduction is further substantiated by the fact that the few remaining bone trabeculae underlying the larger lesions had been so arranged that they paralleled the surface cartilage. The latter structural arrangement was apparently a compensating phenomenon.

The gross structure of the carpometacarpal articulation of the cow seemed equally as important etilogically as the structural rearrangement of the subchondral bone. For this reason, the corresponding joints of the horse were incorporated in this study. Such specimens were not similarly involved even though the majority of them were



## SUMMARY

1. Constant differences in the synovial fluid of the carpometacarpal and astragalotibial articulations of the cow have been described in a previous publication.<sup>1</sup> The finding of areas of degeneration in the articular cartilages of the carpometacarpal articulations of all cattle over 2 years of age would appear to be an adequate explanation of the synovial fluid differences observed.

2. These areas of progressive degeneration in articular cartilage have been studied systematically and the successive changes have been described and illustrated.

3. The development of the carpometacarpal articulations was studied in a series of bovine embryos and calves. The vascular articular cartilages of embryos and calves became avascular before the animals attained the age of 2 years. Pronounced rearrangement of the subchondral bone trabeculae resulted in a relatively deficient bony support of the medial articular cartilage where the degenerative lesions occur.

4. The possible etiological factors of such cartilage lesions have been discussed. It was concluded that they were probably due to repeated trauma in weakly constructed articulations. Deficient subchondral bone support was thought to be an important predisposing factor.

5. The type of cartilage lesion described in this paper is not wholly similar to any of the joint lesions described in human arthritis.

NOTE: We wish to thank the New England Dressed Meat and Wool Company for the material used in this study.

face, where the underlying bone is more directly continuous with the solid cortical shaft. Atrophy and resorption of the bone underlying the cartilage lesions might well take place because the pressure stimulus had been removed.

The histological and gross study showed that the lesions under discussion were not the same as the pathological changes described in any type of human arthritis. The fact that these lesions began as areas of articular cartilage degeneration makes the process more nearly comparable to the degenerative arthritis of Nichols and Richardson <sup>4</sup> than to the proliferative type described by them. They described the degeneration of articular cartilage on one joint surface and a compensatory overgrowth of cartilage and later bone on the opposing joint surface. This compensatory overgrowth of cartilage allowed continued apposition of the involved joint surfaces. In the lesion found in the cow, the degeneration occurred on opposing areas so that continued apposition of the defects was not possible. Nichols and Richardson described thickening and eburnation of the underlying bone. Such changes were never encountered in the joints of cows under study. The degenerative type of arthritis is a disease of older individuals and more common in women. Sex and age (with the exception of the necessary first two years of life) certainly play no part in the incidence of this disease in cattle.

Following the thinning of articular cartilage and rearrangement of bone trabeculae of the metacarpal bones in young cattle, degenerative lesions developed. The sequence of pathological changes leading to degeneration of cartilage appeared to begin with thinning and disruption of the layer of calcified cartilage. Fibrillation of the cartilage matrix followed and was accompanied by replacement of the surface articular cartilage with connective tissue. Disappearance and distortion of cartilage cells was then noted, together with more marked fibrillation of the articular cartilage matrix. Splitting of the fibrillated matrix followed in some instances. Blood vessels grew into the altered cartilage through gaps in the calcified zone, entering from the intertrabecular spaces of the subchondral bone. The altered articular cartilage eventually completely disappeared and varying degrees of subchondral bone resorption followed. The cartilage and bone defects became lined with a vascular connective tissue which resembled synovial membrane to a certain extent.

## DESCRIPTION OF PLATES

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### PLATE 70

FIG. 1. A natural size photograph showing the normal metacarpal articular cartilage of a young calf.

FIG. 2. A photomicrograph of very low magnification showing the entire thickness of cartilage of a 59 cm. bovine embryo. Note the three merging zones of cartilage: (*a*) superficial or perichondrial; (*b*) middle or vascular; (*c*) deep or expanding.  $\times 32$ .

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PLATE 71

FIG. 3. A low power photomicrograph of the metacarpal articular cartilage of a calf. Note the reduced thickness of cartilage (as compared to Fig. 2), the prominent perichondrial border, and the incorporation of three blood vessels by subchondral bone growth. The layer of provisional calcification is well formed.  $\times 37$ .

FIG. 4. A photomicrograph of a normal portion of adult articular cartilage of the cow. One should note the orderly arrangement of cells in lacunar spaces, and the wide zone of calcified cartilage. The subchondral bone trabeculae are broad and very dense.  $\times 84$ .



I



2

PLATE 72

FIG. 5. A metacarpal articular cartilage of a young steer (under 2 years of age). Note the early roughening of articular cartilage in the center of the medial surface. Natural size.

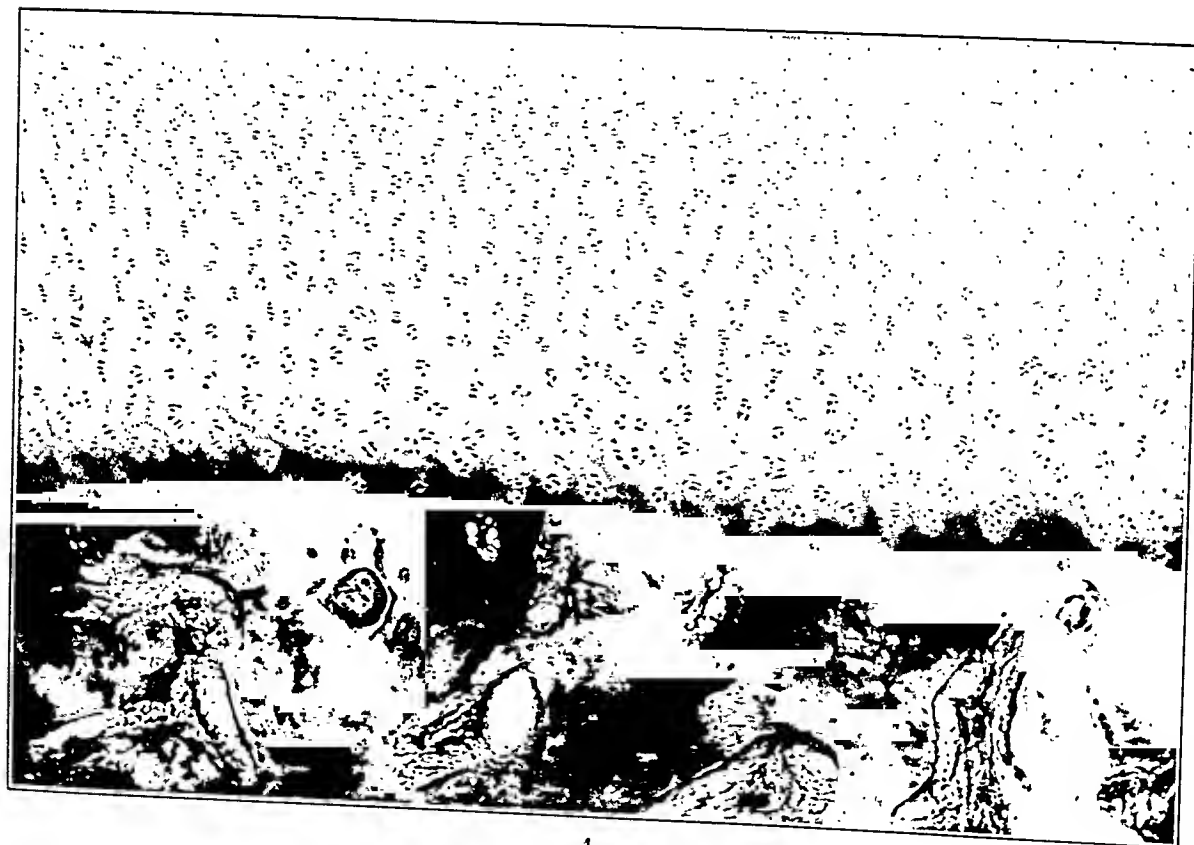
FIG. 6. The medial articular cartilage of this joint shows an area of roughening and thinning which is slightly larger than the defect in Fig. 5. Natural size.

FIG. 7. The central area of the medial articular cartilage is more depressed and the cartilage has been more completely destroyed than in the earlier illustrations. Natural size photograph.

FIG. 8. A natural size photograph of a slightly larger area of degeneration in articular cartilage. This lesion extended down to subchondral bone in a few areas.



3



4

Bennett and Bauer

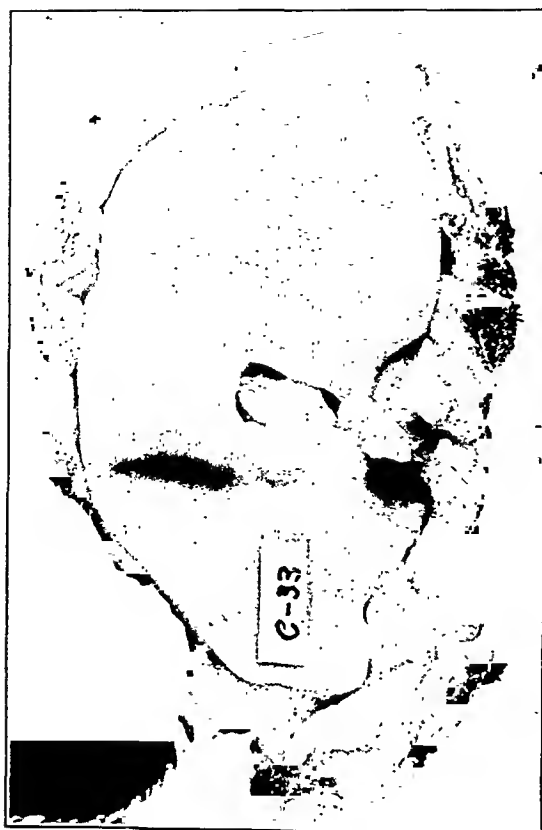
Degeneration of Cartilage in Bovine Joints



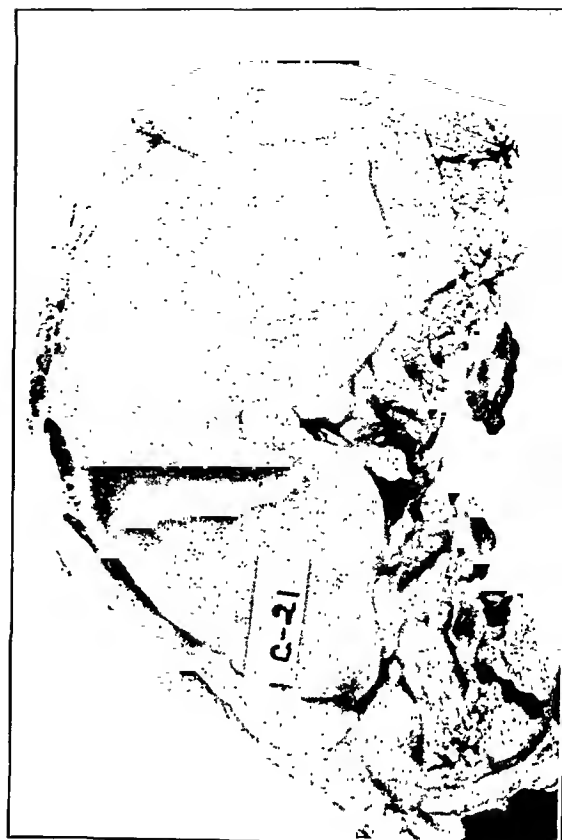
PLATE 73

FIG. 9. A natural sized photograph showing an average sized lesion in articular cartilage which extended into subchondral bone. Note the sharp overhanging margins.

FIG. 10. The articular cartilage surfaces of the right and left metacarpal bones of the same steer. Note the similarity in size, shape and location of the degenerative lesions. Natural size.



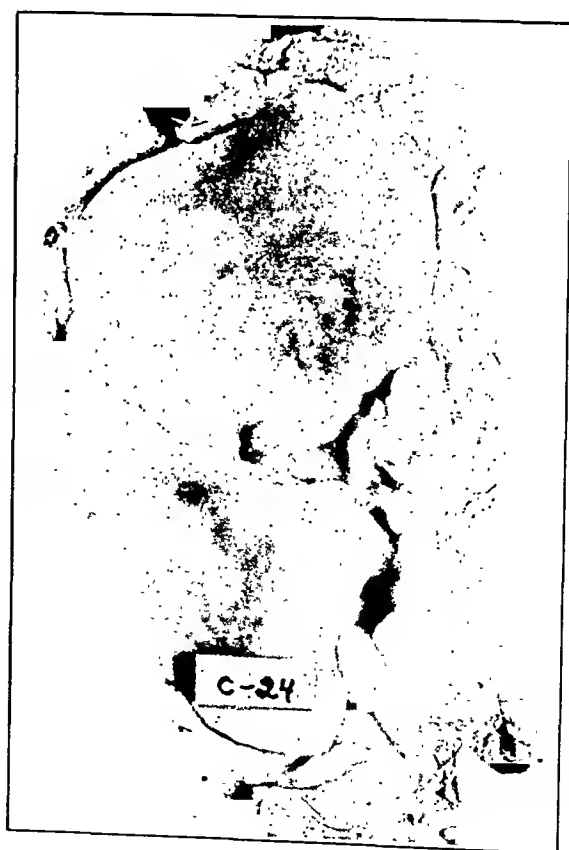
5



6



7



8

Bennett and Bauer

Degeneration of Cartilage in Bovine Joints

PLATE 74

FIG. 11. A carpometacarpal articulation opened in such a way as to show the opposing carpal and metacarpal articular surfaces. The similarity of size and type of degenerative lesion is apparent. Natural size.

FIG. 12. An average sized area of degeneration in cartilage and subchondral bone of an old milch cow. Note the light marginal halo produced by a yellow coloration of the articular cartilage at the margin of the lesion. This feature is peculiar to the lesions in older cattle. Natural size.



9



10

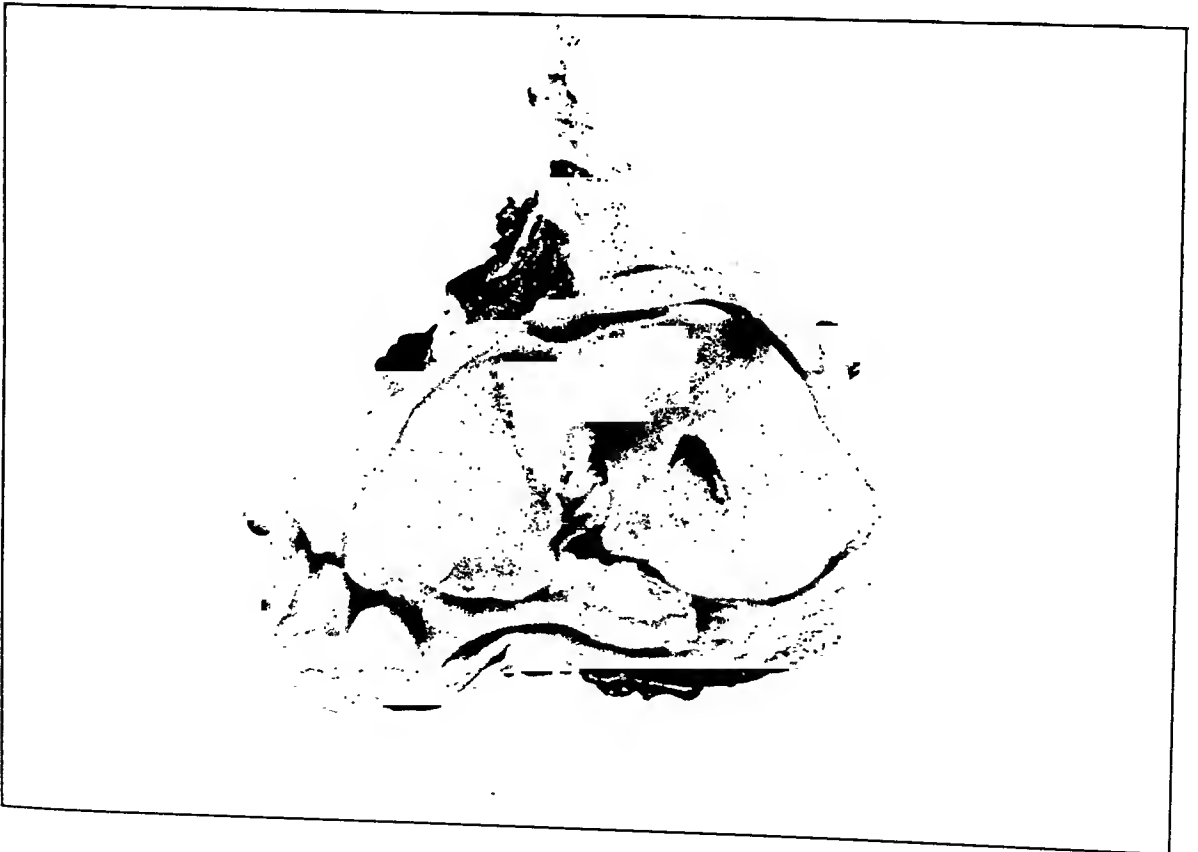
PLATE 75

FIG. 13. Similar lesions on corresponding surfaces of the carpal and metacarpal articular cartilages of an old milch cow. Natural size.

FIG. 14. The articular surfaces of the carpal and metacarpal bones of an old milch cow, showing the striking similarity of the lesion on opposing surfaces. Natural size.



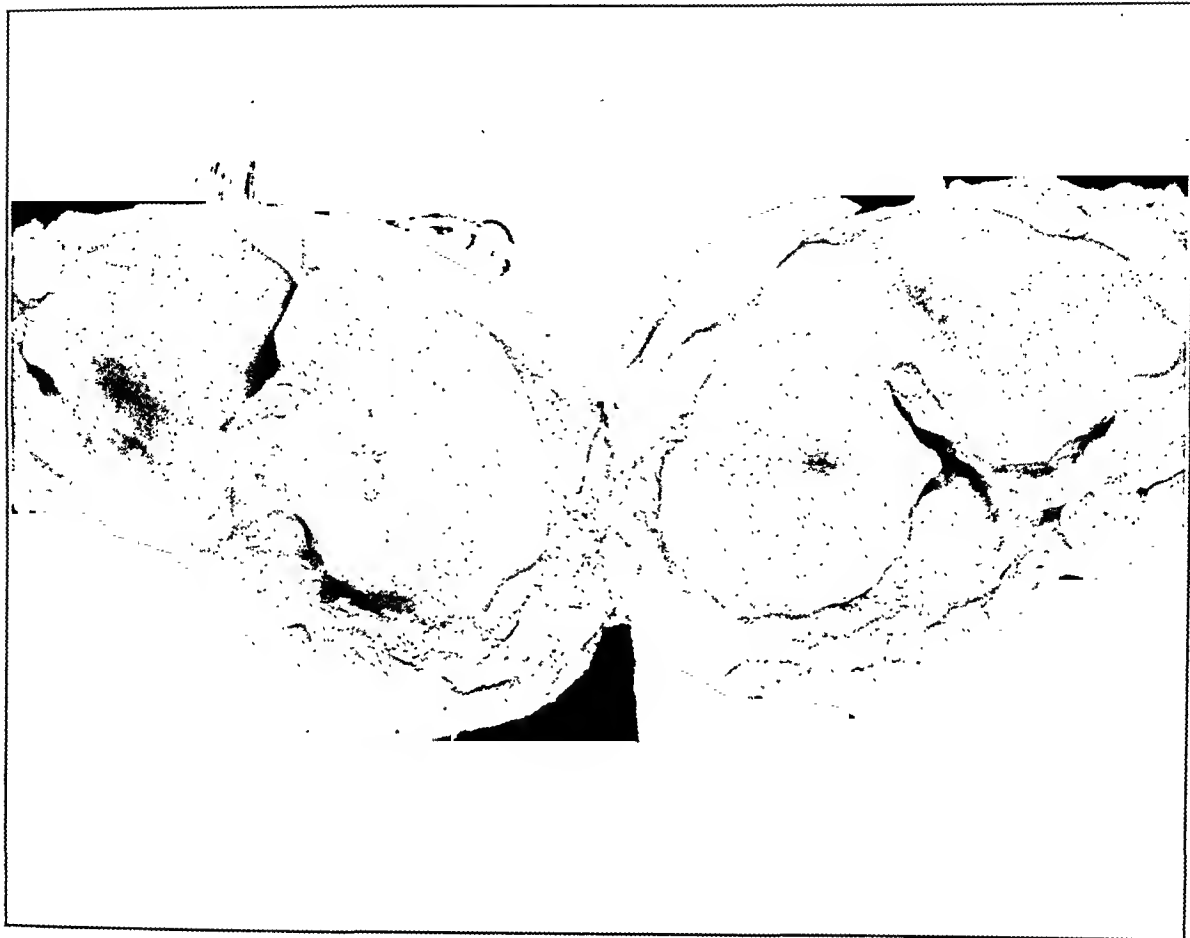
II



12

PLATE 76

- FIG. 15. Early changes in articular cartilage are illustrated in this photomicrograph. The layer of calcified cartilage is thinned out, slightly depressed and completely broken in many places. Beginning distortion of cartilage cells and a surface proliferation of cartilage is illustrated.  $\times 91$ .
- FIG. 16. A very low power photomicrograph showing more extensive distortion of cartilage and proliferation of surface cells. There is much fibrillation and splitting of the articular cartilage matrix. One area of calcification has occurred. Note the depression, thinning and disruption of the calcified layer of cartilage.  $\times 33$ .



13



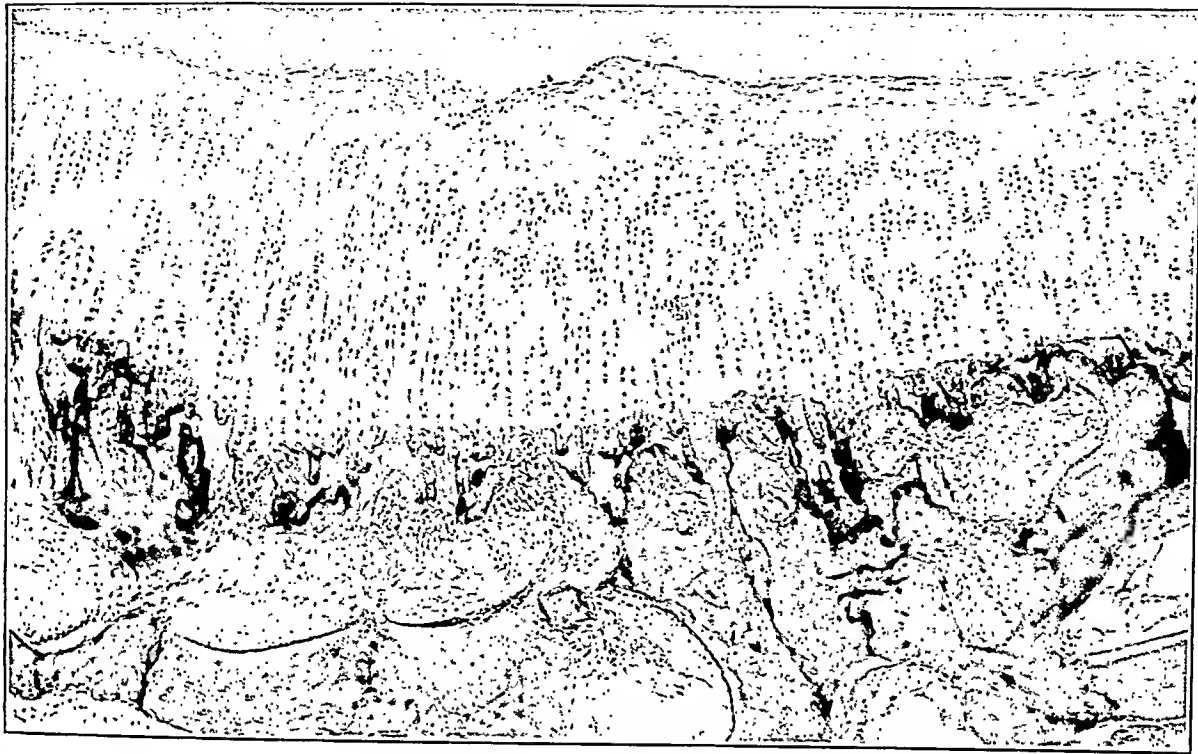
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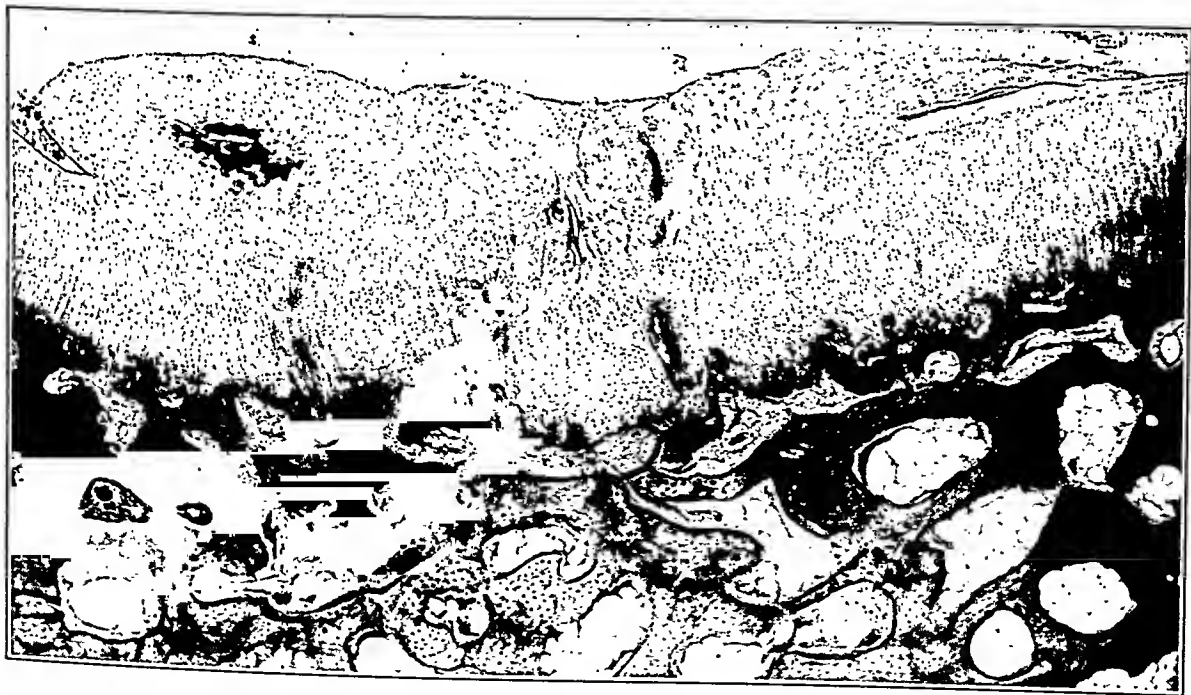
PLATE 77

FIG. 17. Beginning depression of articular cartilage and nearly complete destruction of the layer of calcified cartilage is illustrated in this photomicrograph. Note the alteration of cartilage and its resemblance to fibrocartilage.  $\times 91$ .

FIG. 18. A more advanced metaplasia of cartilage into fibrocartilage. Note the complete absence of the calcified layer and the invasion of cartilage by blood vessels from the subchondral bone.  $\times 91$ .



15



16

PLATE 78

FIGS. 19 and 20. Low power photomicrographs which include one-half of each of the larger lesions from two specimens. Note the abrupt break in articular cartilage, the deep extension of the lesions into subchondral bone and the synovial membrane-like tissue lining the depressions. There is practically no inflammatory cell infiltration in any part of the sections.  $\times 36$ .



17

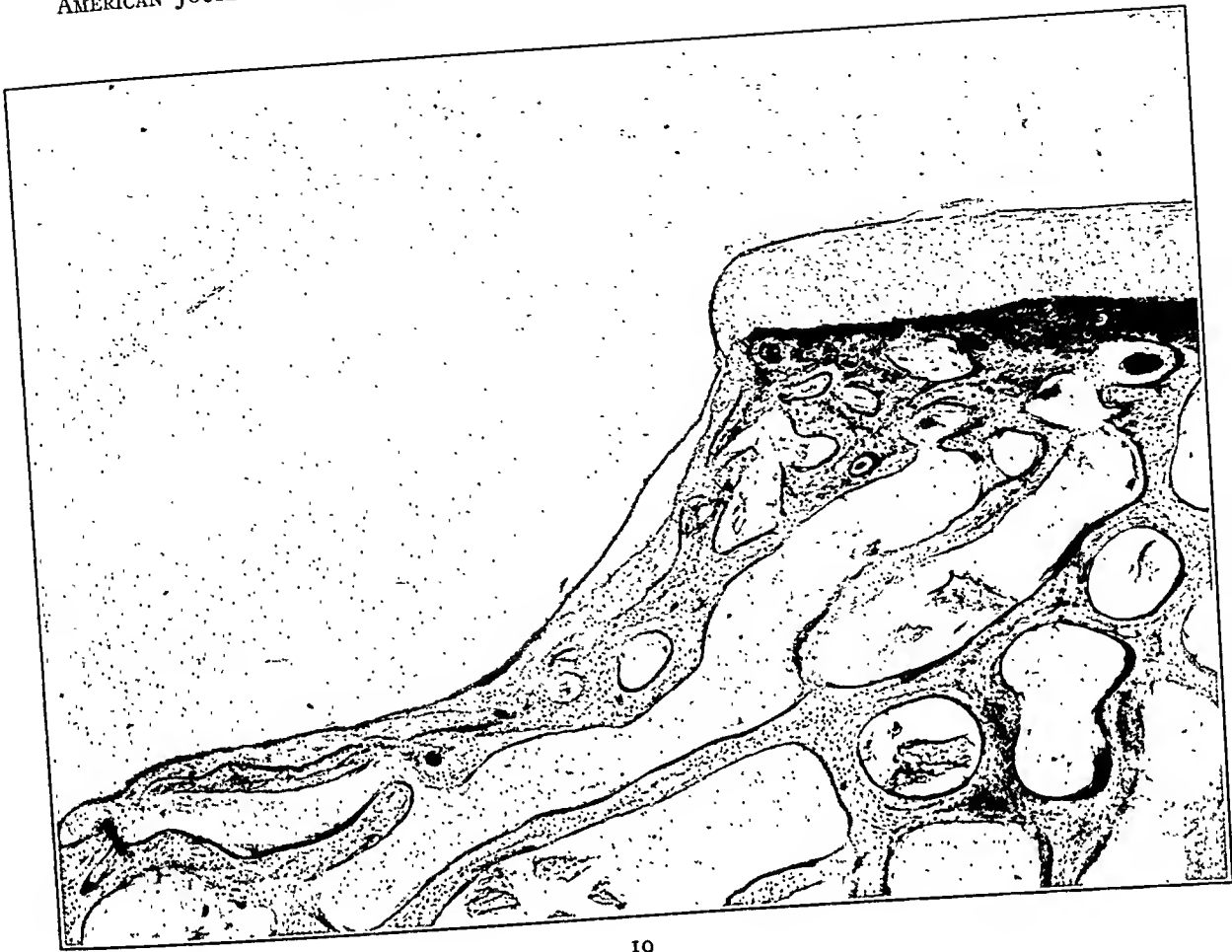


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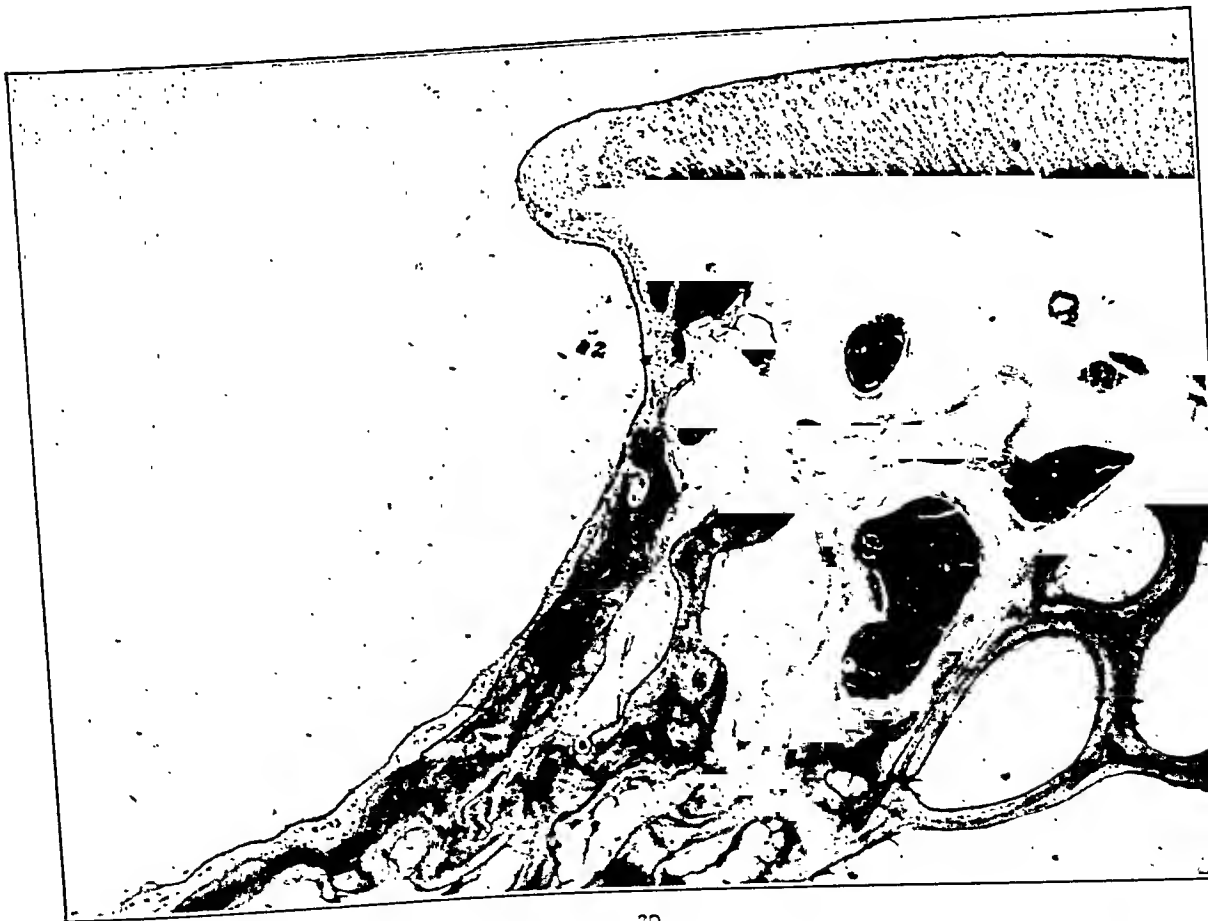
PLATE 79

FIG. 21. Fat replacement of the connective tissue and fibrocartilage which lined the larger and more extensive defects of articular cartilage is illustrated in this photomicrograph.  $\times 91$ .

FIG. 22. One margin of a degenerative lesion in the articular cartilage of an old milch cow. Note the decrease in number of cartilage cells in the more intact cartilage and the intense calcification of the deepest layer of cartilage. The connective tissue lining the degenerated area is largely hyalinized.  $\times 91$ .



19

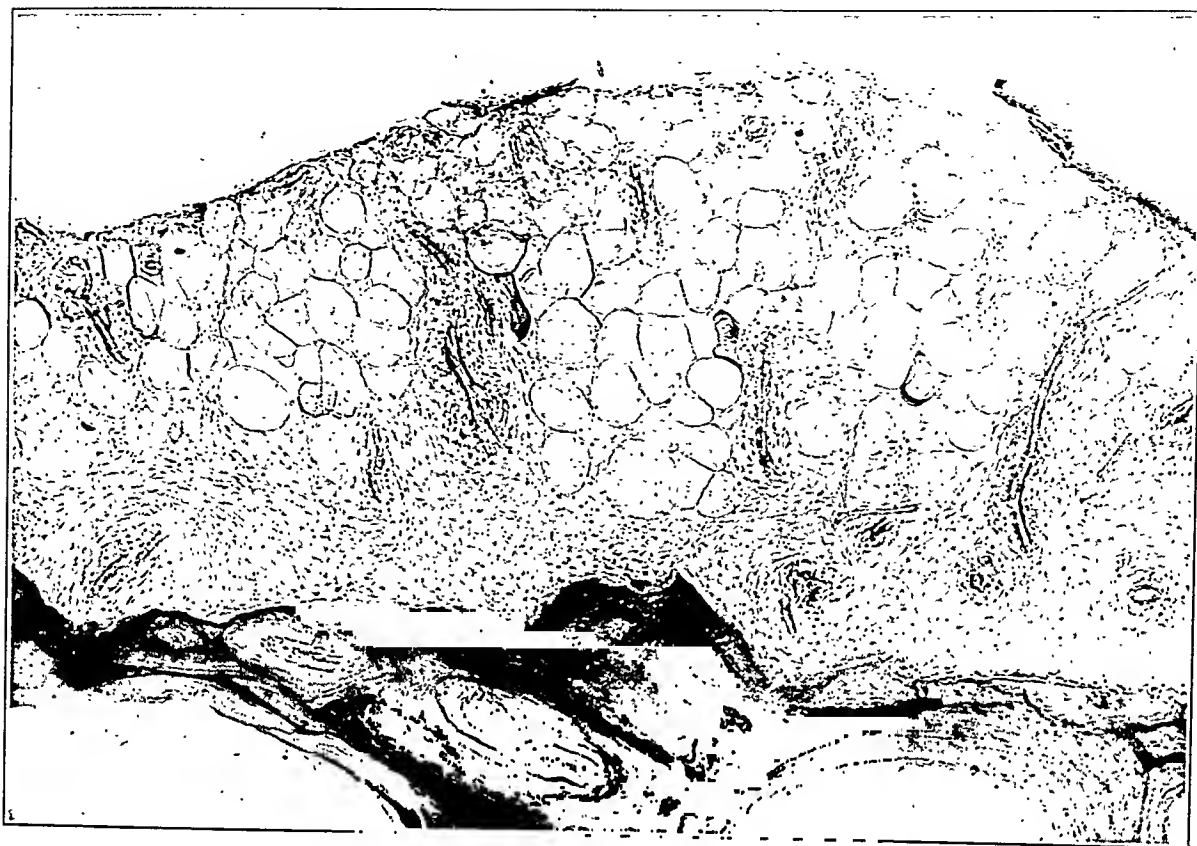


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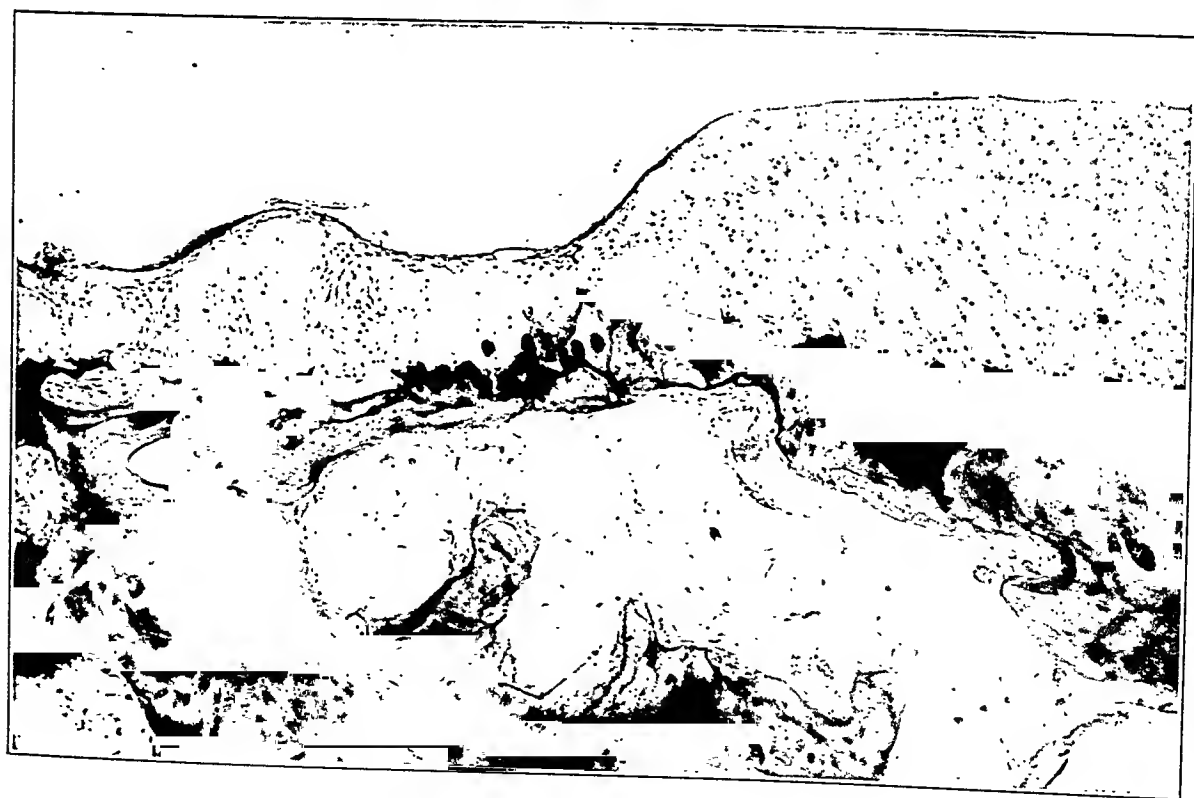
PLATE 80

FIG. 23. A photograph of an entire transverse section (celloidin) showing the evenly distributed subchondral bone trabeculae and thin cortex of the metacarpal bone of a calf.  $\times 2$ .

FIG. 24. A photograph of an entire transverse section (celloidin) of the metacarpal articular cartilage and subchondral bone from a young steer with an average sized lesion of articular cartilage. The difference in the arrangement of the bone trabeculae beneath the medial and lateral articular surfaces and the cartilage defect is clearly shown.  $\times 2$ .



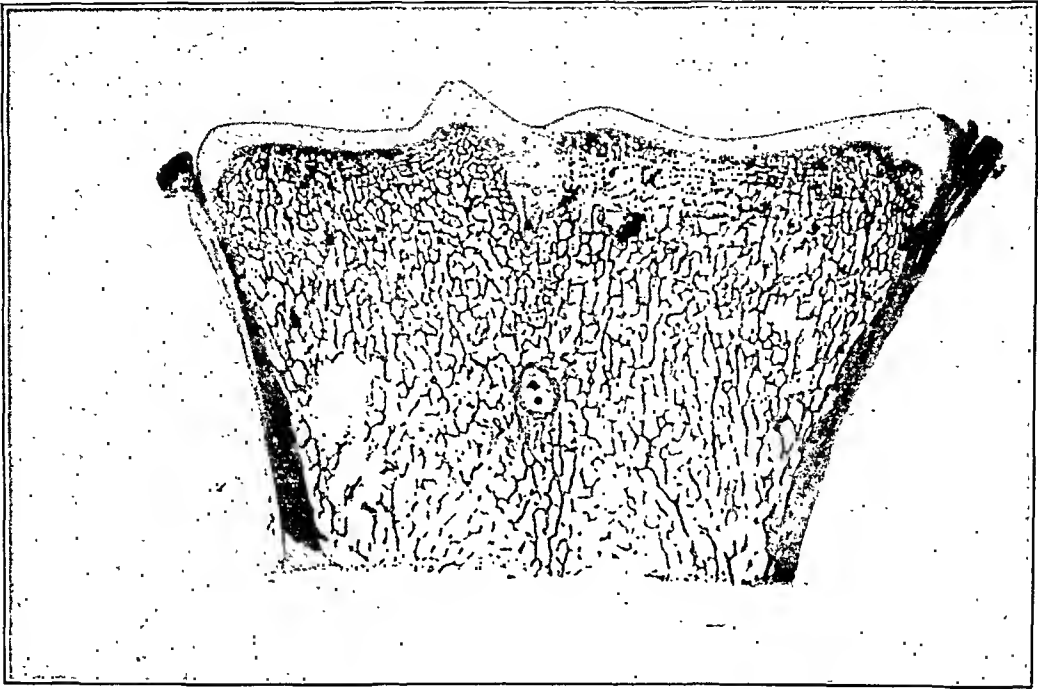
21



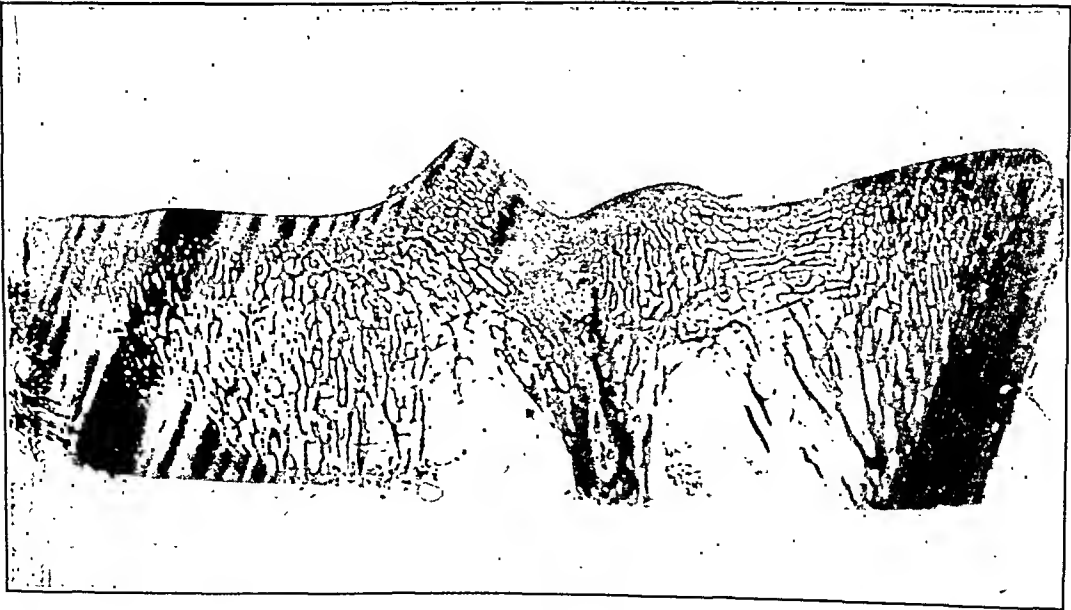
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In general she considered herself to be in good health up to the present illness which had been gradual in onset, starting ten months before. She had been confined to bed for the preceding six weeks. At the onset she was troubled with small areas of soreness and twinges of pain in the anterior thoracic wall.

The ribs were sensitive to pressure and any muscular effort, such as deep breathing or using the pectoral muscles, caused sharp pain. After a time, similar aches and pains appeared in the arms, legs and back. These occasioned much suffering, especially with motion. After a time the muscles became "weak all over" and she was unable to walk or to use her arms except with great effort. Four months before entry, following X-rays of the teeth, three were removed. This was followed by an "osteomyelitis" of the jaw, which had continued to drain. A few weeks before she had developed marked polydipsia and urinary frequency, night and day.

There was marked anorexia. During the preceding month she had vomited several times daily without any definite relation to meals. She had lost 20 pounds in weight since the onset of illness ten months before. A "goiter" had been present for several years.

Physical examination showed a thin, sallow, extremely apathetic woman looking a great deal older than her stated age of 47 years. There was evidence of considerable loss of weight. There was a partial left-sided facial paralysis and left-sided atrophy of the tongue. The musculature everywhere was extremely flabby and all movements were made with considerable effort. In the right mandible, anteriorly, at the site of extraction of two teeth, there was a sinus tract containing a packet of gauze. On removal of the gauze there was an escape of a small quantity of thin purulent material. The breath was uremic. The eye grounds were negative. The right lobe of the thyroid was occupied by a firm rounded mass 5 cm. in diameter. Examination of the heart and lungs elicited nothing significant except that there was pain on deep inspiration. There was some tenderness on pressure on the ribs, anteriorly. There was a scar from the breast operation on the left side. This apparently had been a simple mastectomy. There were no nodules in the axilla or supraclavicularly. The liver edge was palpated two fingers' breadth below the costal margin. Pelvic examination revealed what at first was thought to be a mass, but later was thought to be retroverted uterus. In the right wrist was palpated a bony mass 2 cm. in diameter, obviously arising from one of the carpal bones. All reflexes were present but sluggish. Blood pressure 140/80.

The laboratory examinations at entrance revealed the following: Urine, a very slight trace of albumin with 1 to 3 hyaline casts, 1 to 2 granular casts and 15 to 20 white blood cells per high power field. The urine was negative for Bence-Jones protein. There was a moderate secondary anemia. The white blood cells numbered 10,000. The blood non-protein nitrogen determination showed a value of 56 mg. per 100 cc. The phenolphthalein excretion was 24 per cent in two hours. Wassermann test negative. The blood calcium was 17.3 mg. per 100 cc., the blood phosphorus 4.1 mg. Blood bilirubin 0.8 mg. (van den Bergh).

Following is the report of the X-ray findings, interpreted by Dr. L. B. Morrison:

"The skull shows moderate density and shows some radiolucent areas, two that are at least 1 cm. in diameter, and several areas that are smaller. These are definitely metastatic, the adjoining bone

## METASTATIC CARCINOMA SIMULATING HYPER-PARATHYROIDISM \*

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Upon the basis of experimental and clinical data developed after the discovery of parathormone by Collip,<sup>1</sup> there has been evolved the conception of a clinical syndrome of parathyroid hyperfunction, or hyperparathyroidism. The diagnosis has come to be based upon the following criteria: (1) increased blood calcium, (2) lowered serum phosphorus, (3) increased calcium excretion, (4) widespread rarefaction of bones, and (5) presence of a parathyroid tumor.

Important symptoms are muscular weakness, nausea and vomiting, polyuria and polydipsia, and renal colic.

All of the criteria mentioned have not been present in every reported case; hypercalcemia has been the only constant finding. Thus, in the case reported by Richardson, Aub and Bauer<sup>2</sup> a parathyroid tumor was not found. In Pemberton and Geddie's case<sup>3</sup> there were no demonstrable bony changes. In Wilder's case<sup>4</sup> the calcium excretion was not increased although this may have been influenced by diet and ultraviolet radiation. Barr and Bulger<sup>5</sup> and Hunter<sup>6</sup> present extensive bibliographies which need not be repeated here. Accompanying both articles are abstracts of cases reported up to the past few months.

In the case we wish to report, the interest lies in the decision as to whether we were dealing with a true case of hyperparathyroidism or a simulated condition.

### CASE REPORT

*Clinical History:* The patient, a woman 47 years of age, consulted the Clinic on October 28, 1929, complaining of generalized pains, weakness and vomiting of ten months' duration. The family history was not significant. She had had no infectious diseases. There was nothing to suggest luetic infection. Five years before, a tumor of the left breast had been removed. She had been told at that time that "it might have caused trouble had it not been removed." †

\* Received for publication May 9, 1931.

† A report from the hospital where the operation was performed gave the diagnosis as carcinoma. No microscopic report was obtained.

days, all urine and feces were saved for a three-day period for calcium determination. The data are shown in Table III. A negative calcium balance of 139 mg. or 45 per cent was revealed. Bauer, Al-

TABLE I  
*Serum Calcium and Phosphorus \**

Date	Blood calcium	Blood phosphorus
	mg.	mg.
Nov. 4.....	17.3	4.1
" 5.....	17.6	..
" 11.....	16.6	4.0
" 15.....	15.0	3.0
Operation		
Nov. 16.....	..	..
" 17.....	13.8	3.3
" 18.....	13.7	3.2
" 19.....	13.4	..
" 20.....	13.7	2.5
" 21.....	13.7	2.5
" 24.....	12.9	2.9
Dec. 2.....	14.1	3.1

\* Determinations by Miss H. M. Hunt, New England Deaconess Hospital.

TABLE II  
*Phosphorus and Calcium Intake*

Date	Calcium	Phosphorus
	mg.	mg.
Nov. 8.....	99	169
" 9.....	91	120
" 10.....	136	532
" 11.....	110	149
" 12.....	102	172
" 13.....	95	125

bright and Aub,<sup>8</sup> however, found that when normal individuals were fed on calcium diets (100 mg.  $\pm$  daily) negative calcium balances were manifested. Some of them were equal to the negative balance in this case.

being of normal density. The right mandible shows an area of diminished density just in front of the first molar and down to about the level of the dental foramen.

The ribs show many minute radiolucent areas of the general process compatible with metastases. The third and fourth dorsal bodies are crushing, and they are quite dense. The trachea is crowded slightly to the left, apparently by a small thyroid.

The scapula and upper end of the right humerus show very definite radiolucent areas in which the bone is being destroyed, the adjoining bone being of normal density. The right hand shows radiolucent areas at the base of the fourth metacarpal, and in relation to the os magnum and unciform. The radius and ulna are of normal density, as is the lower end of the humerus.

The fifth lumbar body and both the sacrum and the ilia show radiolucent areas compatible with a general carcinosis. The liver is becoming slightly enlarged. The lungs show no particular changes. The right femur and tibia show no definite changes. The bone is of normal density."

As seen from the above report, Dr. Morrison considered the bone changes compatible with generalized bone metastases. Moreover, he felt that if the condition was due to hyperparathyroidism, there should be no areas of definitely normal bone without calcium deficiency, as there were here.

During the subsequent period of observation the patient's general condition improved somewhat. Vomiting, at first frequent, was finally controlled by intravenous glucose and by careful diet. Under this treatment, also, the blood non-protein nitrogen became lower and the phthalein excretion greater. Anorexia continued, as did the marked weakness and apathy. The high blood calcium determination was checked and persisted at a high level (Table I). The blood phosphorus continued at a normal level. Albright, Bauer, Ropes and Aub,<sup>7</sup> in studying the phosphorus level in the blood, found that parathormone primarily lowers the phosphorus level. If, however, the serum calcium runs above a critical level of about 14 to 15 mg. per 100 cc., the urinary phosphorus excretion falls and the blood phosphorus rises. This may account for the high blood phosphorus levels in this case.

During the following week studies were made of the calcium excretion. A low calcium diet was given (see Table II). After three

proved to the extent that they could be started, her relatives wished to take her home and accordingly these studies were unfinished. She died at home six weeks later. An autopsy was not permitted.

Microscopic examination (No. 6839) of tissue removed from region of parathyroid showed a mass of epithelial cells occurring in clusters, occasionally showing an alveolar arrangement, and embedded in a relatively dense stroma. The cells were moderate in size, polyhedral, with large hyperchromatic nuclei. The cell membranes were ill-defined, the cytoplasm fairly dense and acidophilic but free from granules. Rare mitoses were present, but no abnormal or multiple mitoses were seen. No tumor giant cells were encountered. Near the periphery of the tissue were lymphatics distended with masses of tumor cells. Blood vessels, however, were not involved, except a few of the larger arteries which showed invasion of tumor cells into their perivascular lymphatics. The alveolar arrangement was discernible even within the lymphatics. No secretion was present, however.

The thyroid itself contained an adenomatous nodule 4 cm. in diameter, which had undergone cystic degeneration. Microscopic examination of the wall of the nodule showed invasion in several places by clusters of epithelial cells similar to those described above. Other portions of thyroid tissue showed marked variation in size of follicles, many of which were distended with a considerable amount of colloid, and scattered foci of isolated follicles of fetal type embedded in a rather myxomatous stroma. At various points near the capsule of the gland invasion by tumor cells similar to those described above was seen.

*Microscopic Diagnosis:* Adenocarcinoma, metastatic to thyroid and parathyroid, probably originating from the breast.

## DISCUSSION

Although we felt that we were dealing with a case of generalized carcinomatosis arising from the carcinoma of the breast removed five years before, we were unable to reconcile the persistent hypercalcemia with this diagnosis. Personal experience and a search of the literature showed no similar finding in bone metastases in carcinoma. In addition there was a tumor in the region of the parathyroid. The flaccid muscles with the attendant weakness, vomiting, polydipsia and polyuria could be explained by the hyper-

Although the evidence was not conclusive, exploration for parathyroid tumor seemed warranted. Operation was done November 16. The report follows:

“Ethylene anesthesia. Usual thyroid exposure. The entire right lobe of the thyroid was occupied by a firm mass 5 cm. in diameter. It was exceedingly friable and was adherent to its bed and to the muscles laterally. Lateral to the upper pole on the right was a flat, bean-shaped mass approximately 2 cm. in length, 1 cm. in width and

TABLE III  
*Calcium Intake and Output*

Date	Calcium intake	Calcium output	
		Urine *	Stools †
	mg.	mg.	mg.
Nov. 11.....	110	153	37.5
“ 12.....	102	64	20.8
“ 13.....	95	140	31.0
Total .....	307	357	89.3

Total calcium output ..... 446 mg.  
Total calcium intake ..... 307 mg.  
Negative balance ..... 139 mg.

\* Determinations by Miss H. M. Hunt, New England Deaconess Hospital.  
† Determinations by Dr. Alexander Marble, Massachusetts General Hospital.

3 mm. in thickness. It was wax-brown in color and, except for its size, resembled a parathyroid. A subtotal hemithyroidectomy was done on the right side, excising the tumor at the lower pole and the mass lateral to the upper pole. At the left lower pole was palpated a nodule apparently arising from the gland itself. This was excised.”

During the next few days following the operation, the patient ran a rather stormy course, with persistent vomiting and a rapid pulse. The blood calcium on the day following the operation was 13.8 mg. as compared with 15 mg. on the day before operation. Eight days after operation it was 12.9 mg., the lowest reading. On December 2, fifteen days after operation, it had risen to 14.1 mg. During the post-operative period her condition was not sufficiently stable to carry out studies of calcium balance. By the time her condition had im-



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  9. Thompson, R. L. *J. Med. Res.*, 1911, 24, 291.
- 

## DESCRIPTION OF PLATE

## PLATE 81

FIG. 1. Section from mass of tumor tissue from region of parathyroid. Phosphotungstic acid hematoxylin stain.  $\times 400$ .

FIG. 2. Tumor tissue growing in lymphatic just within capsule of thyroid. Phosphotungstic acid hematoxylin stain.  $\times 400$ .

calcemia. In view of these considerations, together with the widespread bone changes, a diagnosis of hyperparathyroidism was considered possible and was the basis for operation.

The discovery of the malignant nature of the tumor was discouraging to the hypothesis of hyperparathyroidism. While the possibility of a primary malignant tumor of the parathyroid invading the adjacent thyroid and not yet giving rise to metastases has to be considered, there are several facts which militate very strongly against this. The morphology of the tumor is strikingly suggestive of adenocarcinoma of the breast. So far as we know, there are no malignant tumors of the parathyroid which have produced hypercalcemia. The character of the roentgenograms of the bone lesions is far more suggestive of carcinomatous metastases than it is of decalcification resulting from hyperparathyroidism. Finally, the rarity of malignant tumors possessed of highly specialized specific functional activity must be considered.

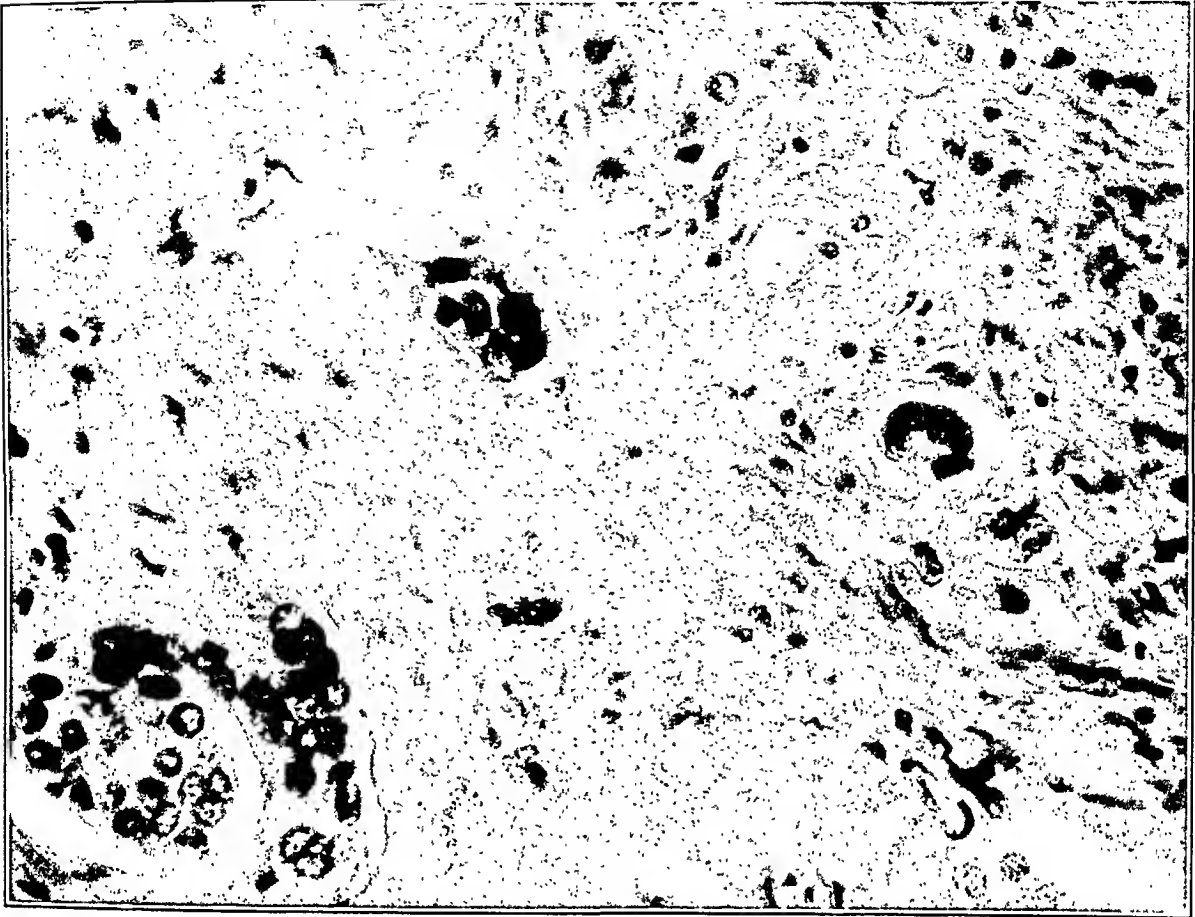
Carcinoma of the breast has been reported by Thompson<sup>9</sup> as metastasizing to the parathyroid glands. He also referred to a case reported by Pepere.

This case is presented as a case of marked hypercalcemia. If marked hypercalcemia is always due to hyperfunction of the parathyroids, we were dealing with a case of hyperparathyroidism. If the hypercalcemia was merely an accompaniment of widespread bone metastases, there are no similar cases recorded in the literature. This report should stimulate further estimation of the serum calcium in metastatic bone carcinoma.

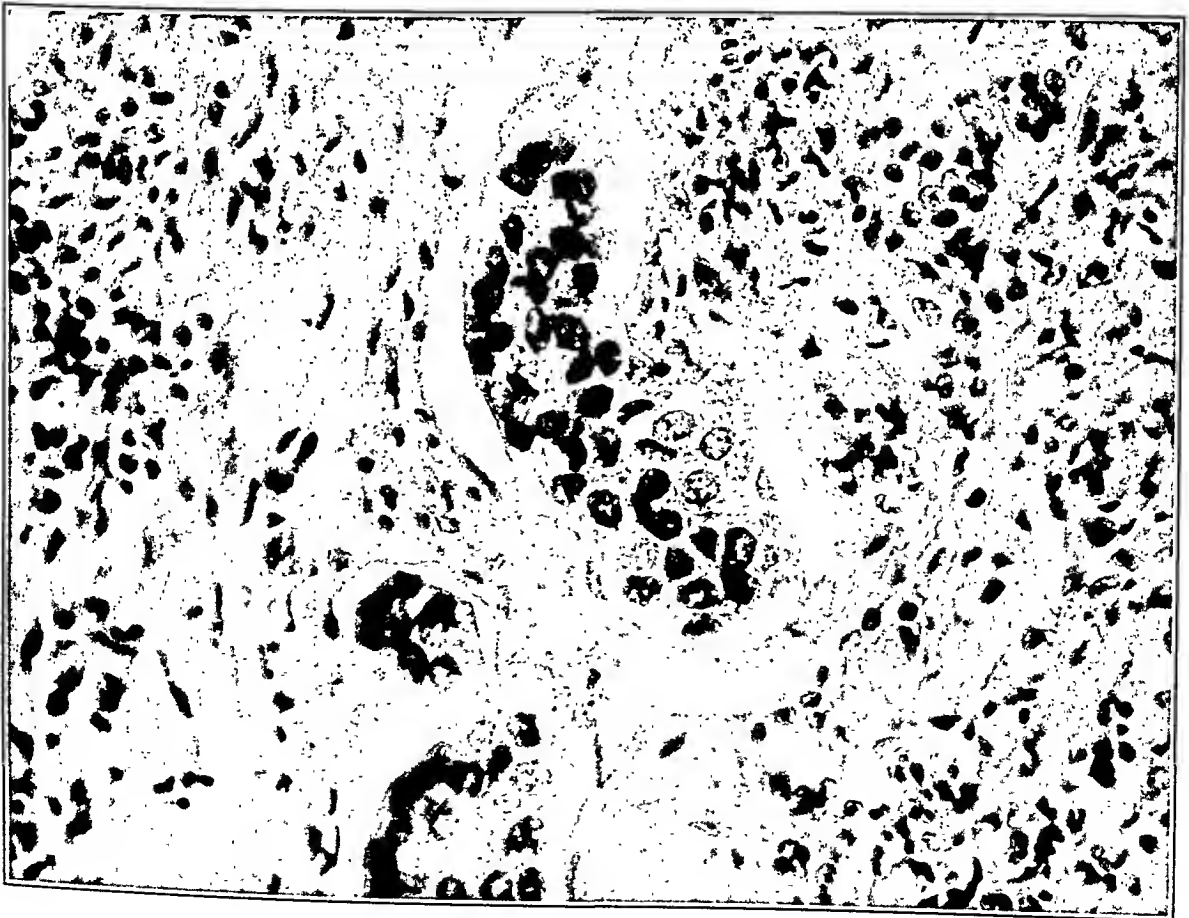
#### SUMMARY

A case with widespread bone changes, hypercalcemia, negative calcium balance and thyroid tumor, is presented. The X-ray appearance of the bones suggested metastatic carcinoma. Examination of parathyroid-like bodies removed at operation showed probable metastatic carcinoma from mammary cancer removed five years before.





1



2

may differ as to the method of origin of an assumed secondary tumor in a given case. Writers may readily misjudge the relative importance of the above mentioned ways for the dissemination of cancer and even question the validity of some of them.

In discussing the origin of peritoneal carcinomatosis, Ewing<sup>1</sup> states: "The most frequent source of these peritoneal growths is the ovarian adenocarcinoma which at any period may rupture its covering and be disseminated through the cavity. Its cells readily become implanted on the peritoneum, producing many miliary or large solid papillary or cystic tumors with a tendency to ascites."

On the other hand Karsner<sup>2</sup> states: "It is supposed that transfer to various parts of the peritoneum of a papilliferous adenocystoma of the ovary is by direct implantation of tumor fragments. This is probably true but cannot be regarded as finally proven because of the possibility that the dissemination is through the subperitoneal lymphatics."

While some pathologists undoubtedly agree with Karsner, Ewing (just quoted), Mallory,<sup>3</sup> MacCallum,<sup>4</sup> Kaufmann<sup>5</sup> and Boyd<sup>6</sup> concede, in their text-books, that certain ovarian tumors metastasize to the peritoneum by cells which escape from the primary growth into the peritoneal cavity and become implanted on the surface of the peritoneum. These pathologists are all well aware of the importance of the lymphatics as a means for the dissemination of cancer. On the other hand the pathologists, already mentioned, who believe in the implantation of cancer on the peritoneum have not described the steps in this process.

I have been able to find in the literature only one description of the implantation of cells from ovarian tumors on the peritoneum, and that by Hertzler.<sup>7</sup> He states: "In papillary serous ovarian cysts often the cyst wall has ruptured allowing the tumor mass to become exposed, particles break off and scatter over the peritoneum and there become implanted. These heal in just as a foreign body does. Their presence excites a reaction on the part of the peritoneum which in turn exudes its plastic material and a fibrin network covers the tumor tissue. Growth then continues." Hertzler's description is an excellent "text" and a true one, but it is not complete. In justice to the subject it should be elaborated and a more detailed description given.

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## IMPLANTATION PERITONEAL CARCINOMATOSIS OF OVARIAN ORIGIN\*

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The purpose of this paper is to present the evidence that implantation peritoneal carcinomatosis of ovarian origin is nothing more than the result of the repair of injuries to the peritoneum caused by cancer cells which have escaped from ovarian tumors into the peritoneal cavity and lodged on the surface of its serous membrane, together with the continued growth of these cells in this situation.

The proof that carcinoma invades its host is absolute, because the direct extension of the former into the tissues of the latter can be definitely demonstrated.

Various ways have been described by which cancer cells, as emboli, may be conveyed from a primary neoplasm to nearby or distant structures and there develop into secondary tumors, *viz.*: the lymphatics, the blood stream, implantation by contact, implantation by cells escaping into cavities lined by serous membranes, implantation on epithelial-lined or covered surfaces and transplantation in the field of operation.

There is abundant support that carcinomata metastasize through the lymphatics. They are the channels by which the majority of these tumors are conceded to disseminate. The chain of evidence is most convincing, but it is broken and therefore the proof is not absolute. The same is true, in varying degree for the other mentioned means for the metastasis of cancer. The evidence in all is at best merely circumstantial. For this reason excellent pathologists

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As emphasized by Mallory,<sup>11</sup> "the underlying principles are the same." We may divide the repair of tissue injury or the healing of wounds into three general stages.

1. *The fixation stage.* Mall<sup>12</sup> showed that the first step in the healing of anastomoses of dogs' intestines is the union of the apposed serous surfaces by fibrin. It is "the basting suture" uniting approximated tissue in wounds and likewise the scaffolding or temporary framework spanning gaps caused by loss of tissue or failure of approximation. Fibrin anchors foreign bodies in wounded tissue and on the surface of the peritoneum. Fibrin also temporarily holds the skin graft in place.

2. *The organizing stage.* Fibroblasts alone, or both fibroblasts and vascular endothelium grow out into the fibrin and make the union of the apposed surfaces of wounds firmer. When there is loss of tissue or gaps caused by failure of approximation, they invade the fibrin in these gaps, giving rise to granulation tissue.

3. *The organized or healed stage.* The fibrin invaded by fibroblasts or replaced by granulation tissue is converted into scar tissue, the final step in the healing of a wound, the encapsulation of foreign bodies, the formation of adhesions and the permanent "taking" of a skin graft.

## MATERIAL AND METHODS OF STUDY

The material which is the basis of the present study was obtained from twenty-five cases of peritoneal carcinomatosis associated with ovarian cancer, operated upon in the gynecological department of the Albany Hospital during the last nine years. It is of very little statistical value because of the small number of cases, the lack of uniformity with which the material was chosen and studied, and the necessarily incomplete study of all lesions possibly present in any operative case. A very important feature of such a problem is the examination of the cellular contents of the fluid present in these cases. This was done in only six instances.

In all but three cases both tubes and ovaries and the uterus were removed at operation, and in some of these also bits of parietal peritoneum, an epiploical appendage, the appendix or a portion of the omentum. But this relatively small amount of material does not enable one to determine the distribution of all the metastases or to

The problem of the implantation of cancer cells on the peritoneum is a combination of the behavior of the peritoneum to small foreign bodies escaping into that cavity and lodging on its serous lining, and of tissue culture — especially the growth of epithelium in skin grafting. Some of the cancer cells are likely to be alive and just as capable of growth as are those escaping into the lymphatics and blood stream. We are not concerned with the site, avenues and means by which foreign material is absorbed from the peritoneal cavity (for a review of the literature of this phase of the subject see an excellent article by Cunningham<sup>8</sup>), but with the fate of foreign material retained in that cavity. Hertzler<sup>9</sup> has reviewed the literature on the results of injecting suspensions of pigment into the peritoneal cavity of the lower animals and includes the results of his own studies on this subject. He states that "this is followed by an increased exudation into the peritoneal cavity. With this increase of exudation there is a sweeping of the particles toward the nooks and dependent portions of the peritoneal cavity and about the great omentum. This may take place in any direction quite as pronouncedly toward the pelvis as toward the diaphragm." The foreign bodies float about until they become anchored on the peritoneum by fibrin, which is transformed into fibrous tissue. Thus the foreign body is eventually covered or encapsulated by the peritoneum.

Neuhof<sup>10</sup> in his monograph, *The Transplantation of Tissues*, reviews the literature on this subject and in describing the histology of autografts of skin (Thiersch graft) states: "There first occurs an exudate of fibrin fixing the transplant in place. This becomes infiltrated with leucocytes and fibroblasts and disappears gradually to be replaced by a richly vascular connective tissue. About fourteen days after transplantation the granulation tissue has changed into an organized membrane."

It can be seen that the reaction of the peritoneum to a dead foreign body is quite similar to that of the tissue receiving the skin graft, except for one very important feature. The tissues of the peritoneum grow over and cover the foreign body while the skin graft remains uncovered. In both instances we are dealing with the repair of injured tissues. The general laws or principles governing the repair of tissue injuries are the same, irrespective of the agent causing the injury, the nature or degree of the latter, its complications or the tissue injured. All these tend to heal after the manner of wounds.



from an ovarian cancer. In lesions involving the peritoneum of the tubes and uterus, a direct extension from the primary tumor, or retrograde metastases through the lymphatics and venous circulation common to these organs, can sometimes be more readily defended.

The cellular contents of the ascitic fluid from six of these cases was studied. A large amount of fluid was centrifugalized, the supernatant fluid poured off and replaced by formalin, thus fixing the sediment. The sediment, when fixed, was removed from the tube, tied in a small piece of gauze and embedded in celloidin. It was then removed from the gauze, mounted, cut and stained with hematoxylin and eosin. In this way we were able accurately to compare the cellular contents of this fluid with those of the primary and secondary tumors.

The oldest patient was 82, and the youngest 38 years of age. Fifteen were over 50 and only two under 40 years of age.

I am unable properly to classify all of the ovarian tumors. Twenty can be grouped as either carcinomatous ovarian cysts or cystic adenocarcinomata. Of these twenty cases, fifteen were bilateral. Of the solid ovarian tumors, only five in number, four were bilateral.

In all instances many peritoneal metastases were present. These were usually most abundant in the posterior cul-de-sac. The frequent infiltration of the peritoneum with cancer in this situation is an important feature in the diagnosis of peritoneal carcinomatosis. Free fluid was present in all cases, and in the majority of them in sufficient amount to cause abdominal distention. Omental metastases were described, in the operative notes, as being present in eighteen cases. In only two instances was the omentum described as appearing normal, while in the remaining five cases no mention was made of its condition, an unfortunate omission. In only a few cases was a portion of the omentum excised for microscopic study. As has been shown in experimental work on the lower animals, the omentum is the great net for picking up foreign particles introduced into the peritoneal cavities of these animals.

To report the results of the microscopic examination of the material obtained from all of the twenty-five patients would at best represent only a small fraction of all conditions actually present in these cases. I trust that a study of the conditions observed in the few cases which have been chosen as the most instructive and repre-

study all types of lesions possibly present. In only two instances was a postmortem examination obtained. In neither of these were the pelvic organs removed at operation. In one an exploratory incision was made, but on account of the great extent of the cancer no attempt was made to remove the primary tumors. In the other a cecostomy was done to relieve intestinal obstruction. In both of these cases metastases were present on the under surface of the diaphragm. At operation one is not able to determine whether or not the diaphragm is involved.

An epiploical appendage was removed at operation in sixteen cases by carefully grasping the base of the appendage by two fine clamps, cutting between them, ligating the stump and immediately placing the severed appendage in formalin without handling it. The appendix, removed in thirteen cases, was also carefully handled in order not to injure its peritoneal surface. Interesting portions of the specimen removed at operation were carefully excised at the close of the operation and placed in formalin. In all instances routine examinations were made of the rest of the specimen removed, but the results of such examinations are unfortunately too often of little value in the study of special problems. The majority of the material was hardened in formalin, embedded in celloidin, and the sections stained with hematoxylin and weak eosin. They were faintly stained with eosin in order to lessen the difficulty in obtaining satisfactory photomicrographs. In many instances serial sections were made in order to determine the exact condition present. This is of the greatest importance, otherwise what appears to be an early implant in a single section may prove to be the advancing edge of a large one, if serial sections are made.

I believe that the study of epiploical appendages and appendices, removed and fixed as just described, is of great value, not only in eliminating trauma and obtaining absolutely fresh material, but also in better determining the possible source of the peritoneal metastases on these structures. It is a short step and an easy one for cancer cells escaping from the ovary into the peritoneal cavity to land, with the assistance of the fluid present in these cases, on the surface of nearby epiploical appendages, the appendix, or even distant structures such as the under surface of the diaphragm. On the other hand, it is a long jaunt, and often over a route difficult to explain, for cancer cells to reach these situations through the lymphatics or blood stream

tical with that of the cooked oatmeal, and also with that shown in the photomicrographs of a similar case reported by Shennan.<sup>13</sup> I subsequently learned that the physician's breakfast, for many years, had consisted of fruit, oatmeal and cream, an egg, toast and a cup of coffee.

CASE 2. The patient, aged 71 years, had an extensive peritoneal carcinomatosis, most marked in the posterior cul-de-sac and in the omentum, associated with cystic adenocarcinoma of both ovaries. A large amount of ascitic fluid was present. The abdominal distention with the physical signs of free fluid, the induration in the posterior cul-de-sac, and a palpable, movable tumor (omental cake) in the midabdomen enabled us to make a correct preoperative diagnosis. At operation, September 18, 1930, a large amount of fluid was obtained, and both tubes and ovaries and an epiploical appendage removed. The ovarian tumors were of about the same size (7 to 8 cm. in their greatest diameters). They were adherent to the posterior layer of the broad ligaments and presented gross evidence that the cancer had extended through their capsules. The histological structure of a carcinomatous metastasis of the peritoneum of the epiploical appendage is shown in Fig. 3. Note its close resemblance to the encapsulated food which had escaped through the perforation of a duodenal ulcer and lodged on the anterior wall of the stomach and the surface of the liver (Case 1, Figs. 1 and 2). The ascitic fluid obtained at operation was centrifugalized and the sediment fixed, embedded in celloidin and cut and stained. The microscopic examination of sections of this sediment showed large masses of mesothelial cells and also clumps of hyperchromatic cells (Fig. 4). The latter were similar to those of the primary tumor and the metastatic growth of the peritoneum of the epiploical appendage. The evidence, strong though only circumstantial, would indicate that the metastatic nodule of the epiploical appendage arose from the encapsulation (implantation) of similar cells becoming attached to the peritoneal surface of the appendage, just as food escaping through a perforated duodenal ulcer became implanted on the peritoneum (Case 1).

CASE 3. The patient, aged 42 years, had extensive peritoneal carcinomatosis, most marked in the omentum, associated with solid alveolar carcinoma of both ovaries. Ascitic fluid was present, but the amount was not as great as in the preceding case. The correct

sentative, together with the illustrations and their legends, may stimulate others to make further and better studies of peritoneal carcinomatosis. It has so influenced me.

### REPORT OF CASES

It is well recognized that foreign material, which escapes into the peritoneal cavity and is not absorbed, becomes encapsulated. The process of encapsulation has been already described. It consists of the fixation of the foreign body by fibrin, the invasion of the fibrin by fibroblasts alone, or by both fibroblasts and vascular endothelium, and finally the replacement of this condition by scar tissue. The results of this process are well shown in the findings of the following case (Case 1).

CASE 1. The patient, a physician, aged 54 years, was suddenly seized with severe abdominal pains, November 21, 1916, while making morning rounds in the Albany Hospital. He remained in the hospital eleven days and was discharged with a diagnosis of acute cholecystitis. This was his first and only acute illness. On June 15, 1918, nearly nineteen months later, he was operated upon after a diagnosis of chronic cholecystitis was made. At operation the gall-bladder was found to be normal, but the distal portion of the stomach, as well as the first part of the duodenum, was bound down by old adhesions. On these adhesions, as well as on the anterior surface of the stomach, were small, opaque nodules resembling metastatic cancer. The appendix was removed, a posterior gastro-enterostomy made, and one of the small nodules on the anterior wall of the stomach was excised. A microscopic examination of the nodule showed it to be encapsulated food (Fig. 1) from an evident perforation of a duodenal ulcer, November 21, 1916. The patient died suddenly December 7, 1922. At postmortem an occlusion of the anterior descending branch of the left coronary artery was found. Small nodules on the peritoneum, which were observed at operation, were still present. One of these, removed at the time of the postmortem from the anterior surface of the right lobe of the liver, is shown in Fig. 2. Food of various kinds, such as bread, peas, corn, oatmeal, fruit and lettuce were fixed in Zenker's solution, cut and stained with the same technique as the nodules removed at operation and at postmortem. The histological structure of the encapsulated foreign bodies just described was iden-

and had only superficially invaded the deeper tissues of these organs (Fig. 8). The ovarian cancer presented the appearance of having been of implantation origin or having arisen from the surface "epithelium" of the ovaries. I believe the latter. Cancer was not found in the lymphatics of the ovaries. The histological structure of the surface of the ovarian tumors, exposed to the peritoneal cavity, demonstrated the ease with which cancer cells could be discharged from these tumors into that cavity (Figs. 9 and 10). Every stage in the reaction of the peritoneum to foreign bodies lodging on its surface, and the encapsulation of the cancer cells (the healing of wounds caused by these) was found (see Figs. 11, 12, 13, 14, 15 and 16). These could all be interpreted as similar stages in the implantation of cancer cells which had escaped from the primary tumor and lodged on the surface of the peritoneum. In the peritoneal metastasis on the epiploical appendage (Fig. 18), the manner of the spread of the cancer over the surface of the appendage is clearly shown, as well as the invasion of its deeper tissues and the possibility of further dissemination of cancer cells to the peritoneal cavity from the surface of exposed portions of the growth. The direct extension of cancer from the peritoneal growths into the underlying tissues was present in many situations (Fig. 16, 18 and 51). In a few situations cancer was found in the peritoneal and subperitoneal lymphatics (Fig. 51). As every metastatic tumor has the same potentialities of invasion and dissemination as a primary tumor in its situation, the presence of cancer in the lymphatics near such a growth could more readily have been derived from cancer near it than from a distant primary tumor. The patient was greatly relieved by the operation, but this relief was only temporary.

CASE 5. Patient, aged 53 years, had a very extensive peritoneal carcinomatosis, most marked in the posterior cul-de-sac and in the omentum, associated with bilateral ovarian carcinomata. A large amount of ascitic fluid was present. A correct preoperative diagnosis was easily made. At operation July 22, 1922, the uterus, both tubes and ovaries, the appendix and epiploical appendage were removed. Ascitic fluid was not saved for a study of its cellular contents. Both ovaries were of about the same size and presented similar conditions. They were both adherent by their lateral surfaces to the posterior layers of the broad ligaments. The histological structure of the surface of these ovarian tumors, exposed to the peritoneal

preoperative diagnosis was considered. At operation, October 15, 1930, fluid was obtained and both tubes and ovaries, the entire uterus and an epiploical appendage removed. The ovaries were of about the same size (6 to 7 cm. in their greatest diameter) and were adherent to the posterior surfaces of the broad ligament and the uterus. The ovarian tumor had invaded the lymphatics of that organ and also had pierced its capsule (see Fig. 5). Clumps of hyperchromatic cells, indistinguishable from those in the ovarian tumor and with an arrangement similar to the latter, were present in the sediment obtained from the centrifugalized ascitic fluid (Fig. 6). Multinuclear giant cells also were present in both situations. Peritoneal lesions in various stages of development were present on the surface of the tubes and uterus. Similar lesions were also present on the peritoneum of the epiploical appendage. One of these is shown in Fig. 7. Clumps of cells, with a histological structure and arrangement identical with those in the ovarian tumor and in the ascitic fluid, are enmeshed in granulation tissue. This represents the organizing stage in the reaction of the peritoneum to injury from foreign bodies lodging on its surface. Cancer was not found in the deeper tissues of the epiploical appendages. The invasion of the lymphatics of the ovary by cancer indicates that it had or might have metastasized through these channels. The exposure of parts of the tumor to the peritoneal cavity, the presence of similar cells in the ascitic fluid, the well recognized reactions of the peritoneum to injuries caused by foreign bodies lodging on its surface, and the inclusion of cancer cells in these reactions indicate that some of the cancer had been disseminated in this way. The patient was greatly relieved by the operation, but this relief was only temporary.

CASE 4. The patient, aged 41 years, had extensive peritoneal carcinomatosis, most marked in the posterior cul-de-sac and omentum, associated with bilateral ovarian carcinoma. A very large amount of ascitic fluid was present. A correct preoperative diagnosis was made. At operation September 26, 1923, both tubes and ovaries, the uterus, an epiploical appendage and a piece of the parietal peritoneum were removed. Unfortunately ascitic fluid was not saved for a study of its cellular contents. Both ovaries were of about the same size and presented similar conditions. They were adherent by their lateral surfaces to the posterior layers of the broad ligaments. The cancer evidently had developed on their lateral and under surfaces

enmeshed in this granulation tissue clumps of cells with an adenomatous arrangement histologically identical with those of the ovarian tumor. We must account for the reaction of the peritoneum to some irritant resulting in the development of granulation tissue, with epithelium-like cells enmeshed in it.

The conditions shown in Figs. 28 and 29 indicate a portal of entry into the peritoneal cavity for both secretions from the ovarian tumors and also malignant cells. Had the patient not been operated upon at this time we would have missed a very important stage in the development of peritoneal carcinomatosis. The patient was seen six months later and presented the physical signs of an extensive peritoneal carcinomatosis, namely — free fluid, marked induration in the cul-de-sac, and a mass in the region of the omentum. Cancer was not found in the lymphatics in any of the sections. I believe that the carcinomata of both ovaries and the body of the uterus were probably independent tumors — an instance of multicentric origin.

CASE 7. The patient, aged 82 years, had an extensive peritoneal carcinomatosis with the greatest involvement in the pelvis and omentum, associated with a cystic adenocarcinoma of both ovaries, the right being the larger and apparently primary tumor. Ascitic fluid was present. The patient was admitted to the Albany Hospital October 6, 1930, with the symptoms of intestinal obstruction. X-rays, after a barium enema, revealed that an obstruction was situated in the sigmoid. An exploratory laparotomy was done with the intention of making a colostomy above the site of obstruction. This was found to be impossible on account of the marked infiltration of the mesocolon with cancer, and a cecostomy was done. The patient died October 21, 1930. At postmortem the following conditions were found: A multilocular adenocarcinocystoma of the right ovary (Fig. 40) which was adherent to the surrounding structures and with gross evidence, in many places, of either an extension of the cancer through the walls of loculi of the cyst or else a rupture of some of these. A marked infiltration of the pelvic peritoneum and organs with cancer was present. The left ovary was only slightly enlarged, but was almost entirely replaced by cancer of the same structure as that in the right ovary, except that the loculi were smaller. The carcinoma in the left ovary had apparently invaded that organ, through its hilum from cancer involving the tissues of the left broad ligament

cavity, demonstrated the ease with which cancer cells might be discharged from the tumor into that cavity (Fig. 20). As in the preceding case, every stage in the reaction of the peritoneum to injury from foreign bodies (cancer cells) lodging on its surface, and the repair of these injuries, was present (see Figs. 21, 22, 23, 24, 25, 26 and 27). All these can be interpreted as similar stages in the implantation of cancer on the peritoneum. Many, but not all, possible phases of the subsequent life history of these peritoneal metastases were found. The direct invasion of underlying tissue (Figs. 19 and 26), necrosis with ulceration (Fig. 19), encapsulation and death (Fig. 19) were present. On the other hand, cancer was not definitely found in the lymph vessels in any of the sections, as in the preceding case. Of special interest is the peritoneal metastasis shown in Fig. 27. It has the appearance of cancer arising from the mesothelium. I believe that it is more likely an implant without encapsulation, similar to a successful skin graft. The patient was greatly relieved by the operation, subsequently had radium and deep X-ray treatment, but died.

CASE 6. Patient, aged 63 years, had a papillary adenocystic carcinoma of both ovaries, and an adenocarcinoma of the mucosa of the body of the uterus, histologically indistinguishable from that in the ovaries; associated with a general peritoneal reaction (granulation tissue stage). There were enmeshed in this granulation tissue occasional clumps of epithelium-like cells with the same histological appearance and arrangement as those of the ovarian tumors. No fully organized peritoneal metastases were found. A large amount of ascitic fluid was present. A correct preoperative diagnosis was not made. At operation June 29, 1923, the entire uterus, both tubes and ovaries were removed, and also the appendix, an epiploical appendage and a piece of the omentum. Ascitic fluid was not saved for a study of its cellular contents. Both ovarian tumors were of the same size and presented similar conditions (Fig. 28). The ovarian tumors were not adherent. The gross appearance of these tumors (Fig. 28), as well as their histological structure (Fig. 29), demonstrates that both cancer cells and any secretion from the growth might readily escape into the peritoneal cavity. Microscopic examination of sections of the tubes (Fig. 32), uterus, epiploical appendage (Figs. 30 and 33) and the appendix (Fig. 31) demonstrated granulation tissue on the peritoneal surface of the same. In a few places there were



briated portion of the tube, which I thought was due to this phenomenon. I believe that they must be of infrequent occurrence as compared with peritoneal metastases arising from the implantation of cells escaping into the peritoneal cavity. It is often difficult to determine the exact method of origin of advanced secondary tumors. The vast majority of the early lesions of peritoneal carcinomatosis which I have studied have presented conditions similar to those caused by injury to the peritoneum from foreign bodies lodging on its surface, and the various stages in the repair of these injuries. When I have observed cancer extending from a metastatic peritoneal growth into the tissues beneath it, or cancer in nearby lymphatics, I have usually considered these a manifestation of invasion and dissemination from the metastatic tumor, rather than an indication of permeation or metastasis from the primary ovarian cancer. This applies particularly to lesions of the peritoneum, of epiploical appendages, appendices and diaphragm, and not to all lesions involving the peritoneum of the tubes and uterus.

From a study of the material which has been presented in this paper, the life history of peritoneal carcinomatous implants of ovarian origin may be divided into the following stages:

1. The escape of cancer cells into the peritoneal cavity from the ovarian tumor.
2. The transportation of these cells to their site of implantation.
3. The reaction of the peritoneal tissue injured by the cancer cells lodging on its surface.
  - (a) Fixation of the cancer cells by fibrin.
  - (b) Organization of the fibrin.
  - (c) The organized implant, the various types and their subsequent behavior.

Cells apparently escape into the peritoneal cavity from a variety of ovarian cancers, not only from cancer arising on the surface of the ovary but also from that starting within that organ and extending through to its surface, and from the rupture of the walls of cystic adenocarcinomata. A study of the histological structure of the portion of these cancers which is exposed to the peritoneal cavity will convince one that this phenomenon must take place (Figs. 5, 8, 9, 10, 20, 28, 29 and 40). This same study indicates that sometimes this may occur early and that the discharge of cancer cells into the peritoneal cavity from some of these tumors may be of frequent

(Fig. 41). Peritoneal metastases of various ages were scattered throughout the abdominal cavity, especially involving the intestinal tract. There was a large metastasis in the omentum. Many small metastases, apparently of different ages, were present on the under surface of the diaphragm (Figs. 42, 43, 44 and 45). The retroperitoneal and mediastinal lymph nodes, which were carefully examined, showed no metastases. With such an extensive involvement of the pelvic organs and the peritoneum and intestinal tract, one would expect to find metastases to the retroperitoneal lymph nodes. All conditions observed in this case can readily be explained by the escape of cancer cells from the primary tumor, their subsequent implantation on the peritoneum and invasion of underlying structures.

### DISCUSSION

I am well aware of the evidence indicating that ovarian cancer, at times, metastasizes through the lymphatics and blood vessels. I have seen cancer cells in these vessels in carcinomatous ovaries and have encountered metastases in the uterus, tubes, and in other situations in patients with apparent primary ovarian carcinoma which could best be explained as having arisen through these channels. I have found metastases in the lymph nodes of only four patients with ovarian cancer. In two of these the peritoneum was free from cancer. In the other two a typical extensive peritoneal carcinomatosis was present. The lymph node containing cancer, in each of the latter cases, was from the groin. It is possible that the cancer cells causing the metastases to the inguinal nodes in these cases might have arisen from the invasion of lymphatics by cancer implanted on the peritoneum about the round ligament or internal ring on that side, rather than from the primary ovarian tumors (bilateral in both cases). I have had the opportunity of studying the abdominal and pelvic lymph nodes of only two patients dying with advanced peritoneal carcinomatosis associated with ovarian cancer. One of these cases is reported in this paper (Case 7). We were unable to find cancer in any of the lymph nodes from these two patients.

I do not deny the possibility of peritoneal metastases arising from an ovarian tumor by way of the lymph or blood streams, but have never seen a peritoneal metastasis in situations other than the fim-

ture. Therefore, the amount of fluid determines, in large measure, the distribution of the metastases. The bottom of the pelvis is exposed to the cellular contents of this fluid earlier and longer than any other part of the peritoneal cavity. It is here that apparently the oldest lesions and the most marked infiltration of the peritoneum with cancer are usually found.

It has been shown in experimental work on the lower animals that a large amount of the foreign material introduced into the peritoneal cavities of these animals becomes enmeshed in the omentum. The omentum of patients with peritoneal carcinomatosis is often markedly infiltrated with cancer.

As is well known, a skin graft will not "take" unless the surface to be grafted is suited for the reception of the former. The cancer cells float about in the fluid and sooner or later some of them lodge on the surface of the peritoneum. Their presence irritates (injures) the peritoneum. This is followed by an exudate and in places a denudation of the peritoneum (casting off of the mesothelium). Fibrin forms on the surface of the injured peritoneum. A study of the very early peritoneal lesions suggests that the cause of the injury inflicted upon the peritoneum by the cancer cells might be more than that of an inert foreign body. Possibly there may be a toxin or enzyme present which escapes from the cancer cells and increases the irritation. It is also possible that the ascitic fluid may sometimes contain this toxin in solution. This might be derived from the cancer cells floating about in the fluid, and possibly also from the primary growth when the contents of a cystic tumor escape into the peritoneal cavity, or from cancer which is exposed to that cavity. The reason for believing that the fluid may contain a soluble irritant is that the peritoneal injury in some cases appears to be much greater than one which can arise from the small number of cancer cells in actual contact with the peritoneum. Next to the cancer cells themselves, fibrin is the most important factor in the life history of these implants (Figs. 11, 12 and 14). It is fibrin which holds the cancer cells in place by splinting them against the denuded peritoneum. The cancer cells may become enmeshed in fibrin which has arched over portions of the peritoneum or dangles from the latter like a fringe. Fibrin is both the temporary framework and the scaffolding of the organized implant, and in large measure determines the form of the latter.

occurrence, or even more or less constant. These cells would usually escape into the pelvic cavity and most frequently lodge in the posterior cul-de-sac. There is often a varying amount of free fluid in the peritoneal cavity, even in patients with essentially normal pelvic organs. I have been collecting this fluid and studying its cellular content for several years and especially the last year. When the amount is small, most of it settles in the pelvis irrespective of the normal posture of the patient. It is often possible at operation on the pelvic organs of women to scoop up with a ladle which I use for this purpose 10 cc. or more of this fluid from the posterior cul-de-sac. Cancer cells escaping from the ovarian tumor would float about in this fluid like the foreign bodies experimentally introduced into the peritoneal cavities of the lower animals, and in time would settle on some portion of the peritoneum. Their presence, in intimate contact with the peritoneum, in some way irritates the latter, with a resulting exudate which increases the amount of fluid already present.

As previously stated, a histological study of the ovarian tumors should convince us that cancer cells escape into the peritoneal cavity from this source. A similar study of the cellular contents of the ascitic fluid obtained from these patients demonstrates in many instances clumps of cells identical in form, arrangement and staining qualities with those of the ovarian tumor, and totally unlike the desquamated mesothelial cells present in the same fluid (Figs. 4, 6 and 49), and also unlike cells found in the ascitic fluid of patients without an ovarian cancer. I believe that the cells which have escaped from ovarian cancers into the peritoneal cavity often continue to multiply in that fluid like bacteria in a fluid culture medium. The clumps of cells frequently are larger than one imagines would break off or be discharged from the primary growth, stain as intensely as those of the primary tumor, and occasionally mitotic figures are present. The fluid arising from the peritoneal irritation caused by cancer cells escaping into the peritoneal cavity is the most important factor in the transportation of these cells. The latter go wherever the fluid goes. The amount of fluid, gravity, and the posture of the patient determine the situation of the fluid and the presence of its cellular contents. When the fluid is small in amount a large part of it remains in the pelvic cavity. When the amount is large it has no difficulty in reaching all parts of the abdominal cavity including the diaphragm, with the patient in the recumbent pos-

by the organ on which it is situated (see the illustrations just mentioned). It manifests itself as a papillary tumor or spreads over the peritoneum with a single layer of cells as though re-covering it. I have designated this uncovered metastasis as an implantation of the skin graft type. Originally the cancer cells might have become attached in a manner similar to a skin graft, or more likely the cancer cells lodging on the peritoneum were first covered by fibrin which did not become organized by fibroblasts and therefore disappeared. It is also possible that even if the fibrin covering it had become organized the growth of the cancer may subsequently have destroyed the latter.

I believe that only occasionally do the cancer cells of an implant die. I have observed a few instances of this. Although encapsulation often checks the growth of these cells so that they may remain dormant for a long time, its efforts are too often futile. There is a constant struggle between the cancer and the tissues of its host, the cancer to invade and its surrounding tissue to check that invasion. Polypoid implants with a sessile base may gradually flatten. This is caused by the invasion of the structures beneath it, the lateral expansion of the tumor, and the attempt of the surrounding tissue to encapsulate it (compare Fig. 16 with Fig. 51, and Fig. 54 with Fig. 56).

As a general rule cancer implanted on the peritoneum has the same potentialities of invasion and dissemination as a primary tumor. It invades the peritoneum and underlying structures (see Figs. 16, 18, 19, 20, 26, 38, 39, 41, 45, 48, 51, 52, 53, 54, 55, 56 and 58). It gains access to lymph vessels (Figs. 51, 55, 56, 58 and 59) and probably metastasizes through them. The conditions shown in Figs. 18, 24, 27, 46 and 47 indicate that cancer cells escape into the peritoneal cavity from such implants as well as from the primary tumor, and may give rise to secondary implantations of cancer on the peritoneum.

Patients with ovarian carcinoma usually do not die from the primary growth, but from its implants by the direct extension and dissemination of cancer cells from these.

Bilateral ovarian cancer was found in nineteen of the twenty-five cases of peritoneal carcinomatosis. The question naturally arises, were these ovarian cancers independent tumors or was one secondary to the other? When the tumors are of the same or nearly of the

The fibrin becomes organized by fibroblasts growing out through breaks in the surface of the injured peritoneum and invading the fibrin (Figs. 11, 12 and 14). Sometimes organization apparently occurs with the assistance of fibroblasts alone. In other instances vascular endothelium also invades the fibrin, which with the fibroblasts forms granulation tissue (Figs. 15, 30, 31, 33 and 34).

The fully organized implant arises from the transformation of fibrin invaded by fibroblasts, or of granulation tissue into connective tissue. It is the result of the repair of the peritoneum injured by cancer cells lodging on its surface (the scar of the peritoneal wound). Its form is determined by many factors — the condition of the surface of the peritoneum at the time of the fixation of the cancer cells; the amount of fibrin and its arrangement (the architecture of the scaffolding and temporary framework); the situation of the cancer cells in this design, whether beneath the fibrin, enmeshed in it or on its surface; the type of the organization of the fibrin (whether by fibroblasts alone or both by fibroblasts and vascular endothelium), and its age and the activity of the cancer cells.

Often the mature implant consists of cancer cells embedded in the thickened peritoneum (Figs. 3, 13, 24, 26, 44, 45 and 48). In other instances it resembles a polyp wholly or partially encapsulated; the polyp may be sessile and when so the base may be solid (Figs. 16, 23, 38, 39, 51, 54 and 56) or fenestrated (Fig. 23); occasionally the polyp is attached to the peritoneum by a very slender pedicle (Figs. 17 and 37). Implantations in adhesions frequently occur (Figs. 7, 33, 34, 35 and 36). All of these forms, just described, are the results of the repair of the peritoneum injured by cancer cells lodging on its surface.

There is one type of peritoneal cancer found in patients with peritoneal carcinomatosis for which a metastatic origin might be questioned, and that is the non-embedded or non-encapsulated growth. This is attached to or spreads over the surface of the peritoneum like a primary tumor which had arisen from the mesothelium in this situation (an instance of the multicentric origin of cancer). In my experience it is of infrequent occurrence as compared with the other types already described. Excellent examples of this type of growth are shown in Figs. 24, 27, 42, 46 and 47. It is not peculiar to any one variety of ovarian cancer and is found with other types of implants in the same patient. Its development apparently is not determined

tions of cancer actually arise from the implants? Apparently they can.

Does the peritoneum of patients with peritoneal carcinomatosis actually develop a relative immunity to the implantation of cancer on its surface? I believe that this occurs in some instances.

What can be done to increase this immunity and likewise the encapsulation of the cancer cells, and retardation of the growth of these cells? Will deep X-ray therapy and radium accomplish this?

Is the removal of the omentum of any value? These and many other questions have occurred to me while studying this subject.

### CONCLUSIONS

Implantation peritoneal carcinomatosis arises from the repair of injuries to the peritoneum caused by cancer cells which have escaped into the peritoneal cavity and lodged on the surface of its serous membrane, together with the continued growth of these cells in this situation.

The various stages in this repair, as well as the laws governing the same, are similar to those encountered in the repair of tissues injured by foreign bodies, and in the taking of skin grafts — namely, the healing of wounds.

The histological structure of these implants varies with the reaction of the peritoneal tissues before and after the fixation of the cancer cells and the activity of the latter.

As a result cancer becomes embedded in the peritoneal scar, encapsulated on its surface, enmeshed in adhesions, or like the epithelial growth of a successful skin graft spreads over the peritoneum without encapsulation.

The malignant cells of these metastatic tumors possess the same potentialities of invasion and dissemination as those of a primary cancer.

NOTE: The laboratory work on this paper was greatly facilitated by the technical skill and care of Miss Isabel Peck.

The illustrations of the gross specimens and the coloring of the photomicrographs were made by Mrs. M. R. Marden, and the photomicrographs by Mr. James A. Glenn. These I thank for their interest and coöperation.

same size, one is tempted to believe that they might be independent tumors (multicentric origin). When there is a marked difference in their size, one may have been derived from the other, or the development of the cancer in one of the ovaries may have been deferred. A study of the peritoneal surfaces of malignant ovarian tumors demonstrates implantation-like lesions in various stages of development which have a histological structure identical with the peritoneal implants in other situations in the same patient.

For this reason I believe that sometimes one ovarian cancer may be derived from the other by the implantation of cancer cells on its surface. I have seen only one instance in which the cancer of the second ovary was very small. It presented the histological structure of an implant of the skin graft type (not encapsulated), and therefore was indistinguishable from a cancer arising from the surface epithelium of the ovary.

The infiltration of the omentum with cancer is usually so great that my studies of its development in this situation have been unsatisfactory. I would expect it to present the same stages as implantations elsewhere in the peritoneal cavity.

It is obvious that there are many problems of both scientific interest and clinical importance associated with implantation peritoneal carcinomatosis of ovarian origin.

Why are patients apparently benefited by the removal of the pelvic organs? It is true that the relief is usually only temporary. All but three of the twenty-five patients mentioned in this paper are dead. The three living ones were operated upon within a year and a half. By removal of the primary tumors we often remove only one source of the dissemination of cancer cells into the peritoneal cavity. It is true that the majority of implants are encapsulated, but some are not, and even in those which are, the cancer, by direct extension, often pierces the capsule.

Do cancer cells escaping into the peritoneal cavity actually multiply like bacteria in a fluid culture medium? I believe they do. How long will they live in this medium without implantation? Is the irritation of the peritoneum by these cells solely that of a foreign body or is there some toxin in these cells which adds to the injury? If there is a toxin, may it not also be in solution in the ascitic fluid?

Is the malignancy of the cancer cells in the implant greater or less than that of those in the ovarian tumor? Can secondary implanta-



## DESCRIPTION OF PLATES

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### PLATE 82

FIG. 1. Photomicrograph of encapsulated food found, at operation, on the anterior wall of the stomach of a patient who had a perforation of a duodenal ulcer, one and one-half years before (Case 1). The foreign body is entirely encapsulated and strands of connective tissue, like trabeculae or the stroma of a cancer, have developed between portions of it. This represents the results of the repair of injury to the peritoneum caused by a particle of food lodging on its surface.  $\times 25$ .

FIG. 2. Photomicrograph of food embedded in the capsule of the anterior surface of the liver, obtained at postmortem (Case 1), four and one-half years after the operation mentioned in Fig. 1. The clinical history of the case indicates that this food escaped into the peritoneal cavity six years before death. The histological structure of the food in this section is identical with that shown in Fig. 1 and also with that of cooked oatmeal fixed in Zenker's solution, cut and stained with hematoxylin and eosin, as was the encapsulated food shown in this and the preceding illustration.  $\times 25$ .

FIG. 3. Photomicrograph of a metastatic cancer of the peritoneum of an epiploical appendage from a patient with peritoneal carcinomatosis associated with bilateral ovarian cancer (Case 2). Histologically it is in many ways identical with the final stages in the healing of the wounds of the peritoneum caused by food escaping into the peritoneal cavity, shown in Figs. 1 and 2. It differs from the latter in that the cells of this encapsulated foreign body have grown and invaded its capsule. It represents either cancer arising in the peritoneum independent of the ovarian cancer (multi-centric origin) or, if derived from the latter, it must have been from cancer cells transported to this situation through the blood or lymph vessels, or by the encapsulation (implantation) of cells escaping into the peritoneal cavity from the ovarian tumors. Cancer was not found in the lymphatics of the appendage.  $\times 25$ .

FIG. 4. Photomicrograph of a section of the sediment from centrifugalized ascitic fluid obtained from Case 2. I believe that the paler cells were derived from the mesothelium but the clumps of hyperchromatic cells, similar to those in the ovarian tumors, as well as those in the metastatic nodule shown in Fig. 3, might have escaped into the peritoneal cavity from the primary tumors. Could the condition shown in Fig. 3 possibly have arisen from the repair of injury to the peritoneum of the epiploical appendage by similar cells lodging on its surface, just as food escaping into the peritoneal cavity became encapsulated on or embedded in the peritoneum (Figs. 1 and 2)?  $\times 60$ .

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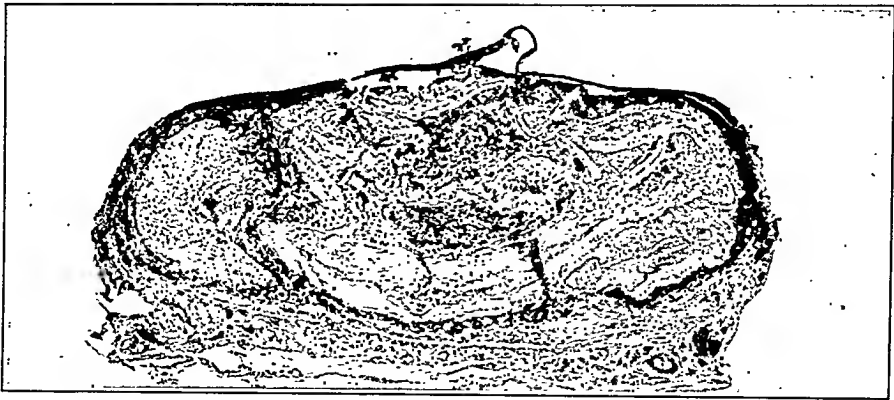
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### PLATE 83

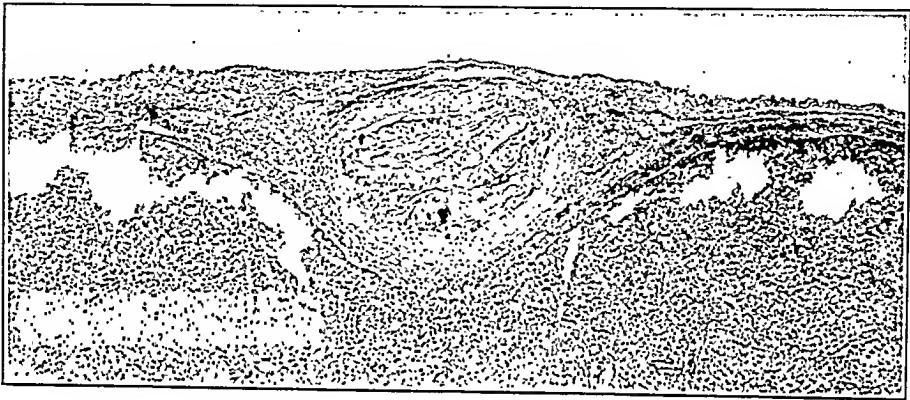
FIG. 5. Photomicrograph of a section through a portion of the surface of an ovary of a patient with peritoneal carcinomatosis associated with bilateral ovarian cancer (Case 3). It is conceivable that cancer cells might easily escape into the peritoneal cavity from such an area. If so we would expect to find them in the ascitic fluid (Fig. 6). Should any of these cells wound the peritoneum we would expect to find evidence of such an injury (see Fig. 7).  $\times 60$ .

FIG. 6. Photomicrograph of a section of the sediment from the centrifugalized ascitic fluid obtained from Case 3. Clumps of hyperchromatic cells are present, with an appearance and arrangement identical with those shown in Fig. 5. Multinuclear giant cells are also present as well as clumps of smaller and paler cells similar to the mesothelial cells shown in Fig. 3. Were the larger, hyperchromatic cells derived from a metaplasia of the peritoneal mesothelium or from cancer cells discharged from the ovary shown above? Is there evidence that similar cells might have injured the peritoneum as foreign bodies injure this structure?  $\times 130$ .

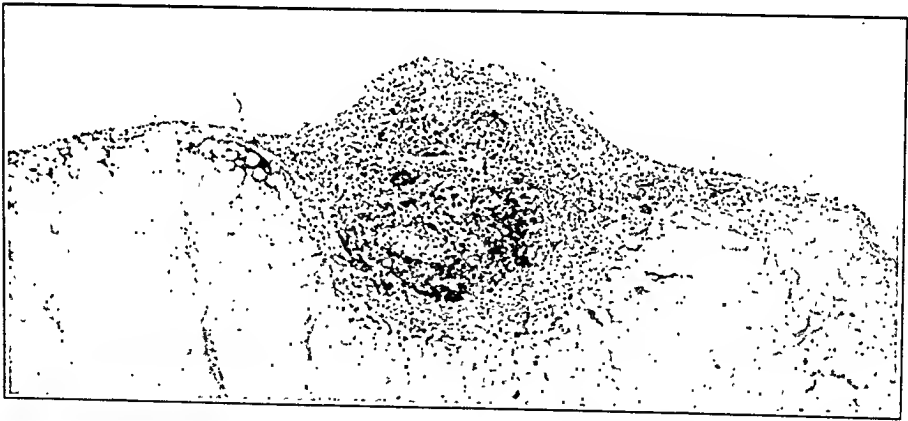
FIG. 7. Photomicrograph of a section through the surface of an epiploical appendage (Case 3). Granulation tissue, a well recognized reaction to tissue injury, has arisen on the peritoneal surface of the appendage and enmeshed in this tissue are clumps of cells identical in appearance and arrangement with those of the ovarian cancer (Fig. 5), as well as with the cells of the sediment from the centrifugalized fluid (Fig. 6). The condition is similar to the organizing stage in the repair of injury to the peritoneum from foreign bodies escaping into the peritoneal cavity. Might it not also represent a similar stage in the repair of a like injury to the peritoneum caused by cancer cells which had escaped from the ovarian tumor, as indicated in Figs. 5 and 6?  $\times 60$ .



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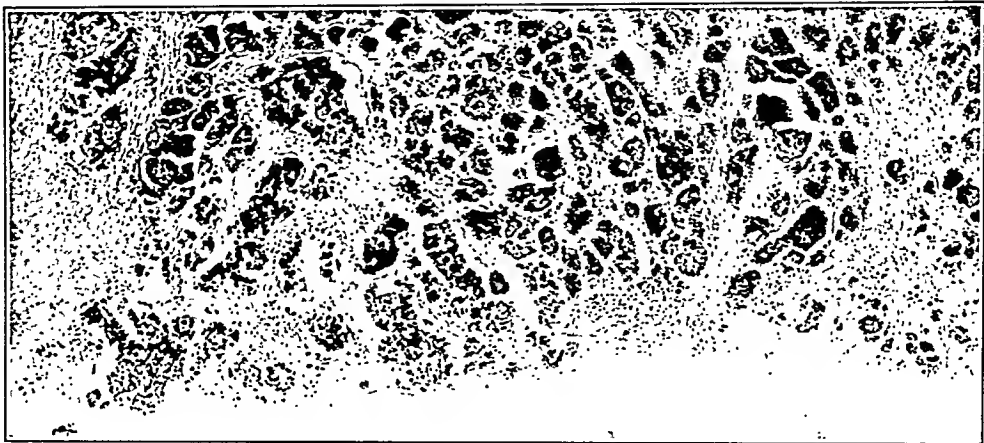


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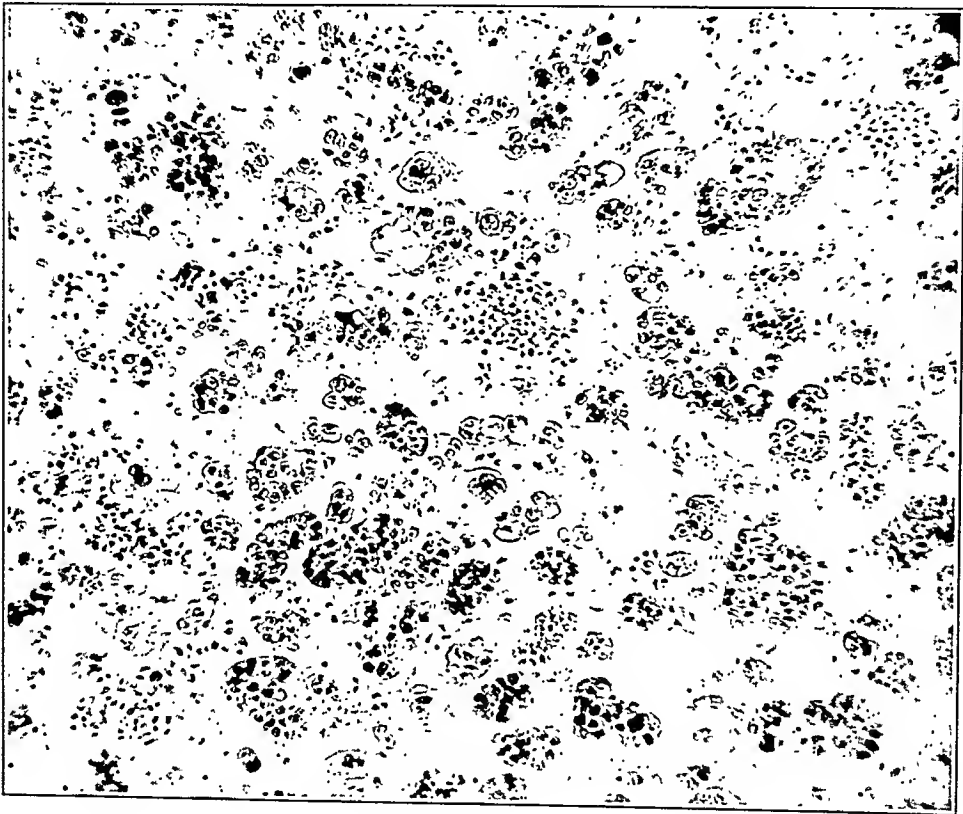
PLATE 84

FIG. 8. Photomicrograph of a cross-section of the right ovary from a patient with peritoneal carcinomatosis, associated with bilateral ovarian cancer (Case 4). The cancer has invaded the ovary from its peritoneal surface and is most marked on the lateral surface which was fused with the posterior layer of the broad ligament. The under surface of the ovary was free and here cancer can be seen arising from, or replacing the surface epithelium. (See Fig. 9.)  $\times 5$ .

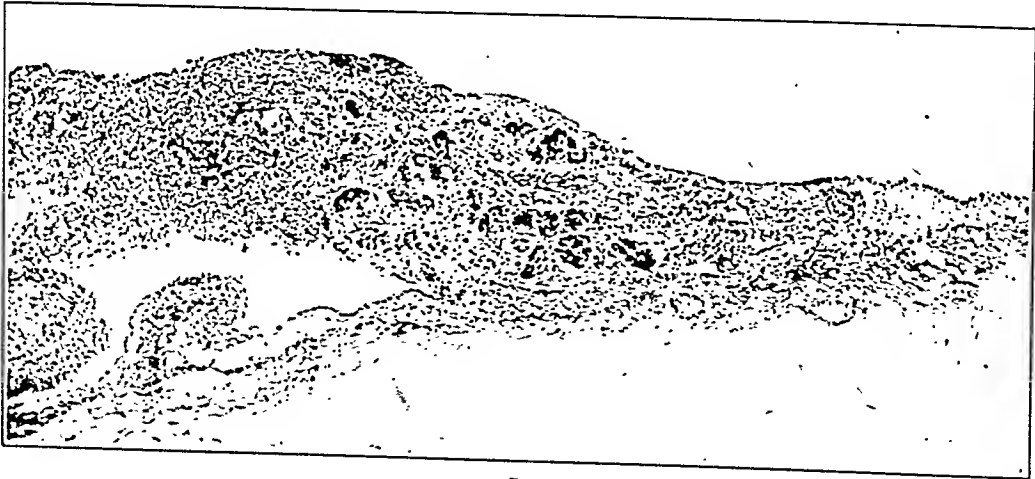
FIG. 9. Photomicrograph of a portion (a) of the under surface of the ovary shown in the preceding illustration. The origin of the cancer from the surface epithelium or the replacement of the latter by the growth is well shown. One can understand how cancer cells might easily escape into the peritoneal cavity from this portion of the growth. Unfortunately ascitic fluid, in this case, was not saved for a study of its cellular contents.  $\times 60$ .



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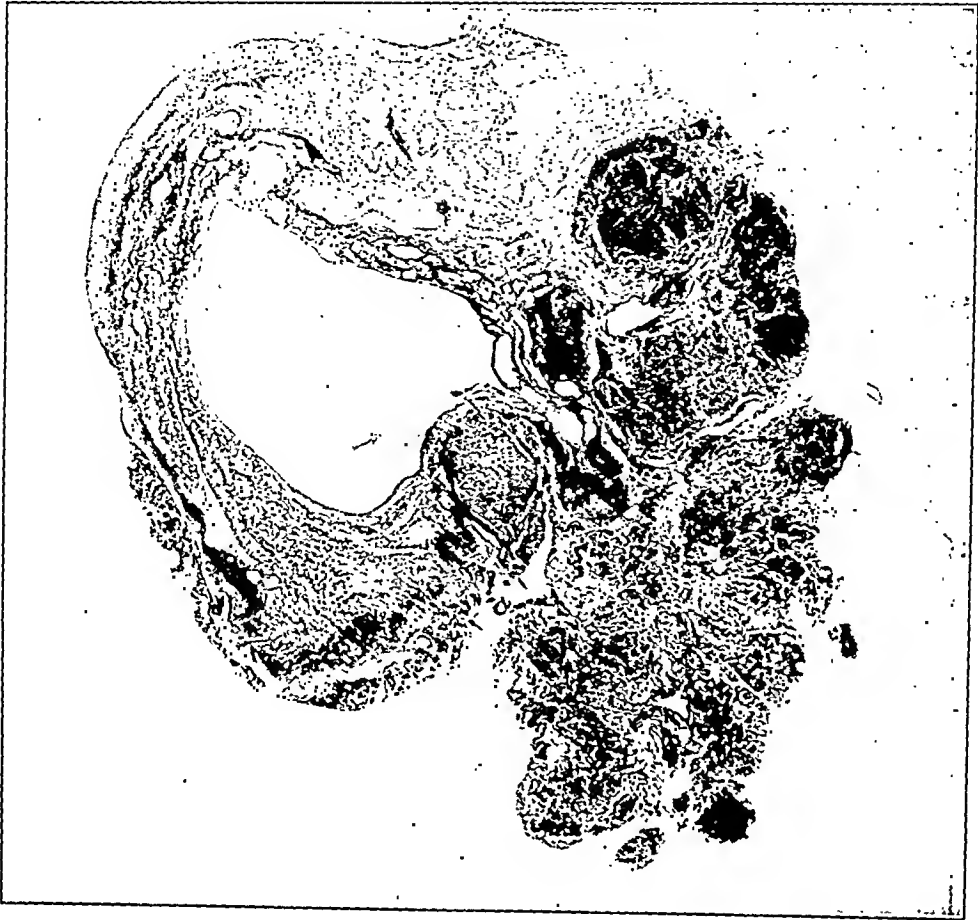
Implantation Peritoneal Carcinomatosis

FIG. 10. Photomicrograph of the surface of the cancer shown in the preceding illustration, again emphasizing the ease with which malignant cells might escape into the peritoneal cavity.  $\times 130$ .

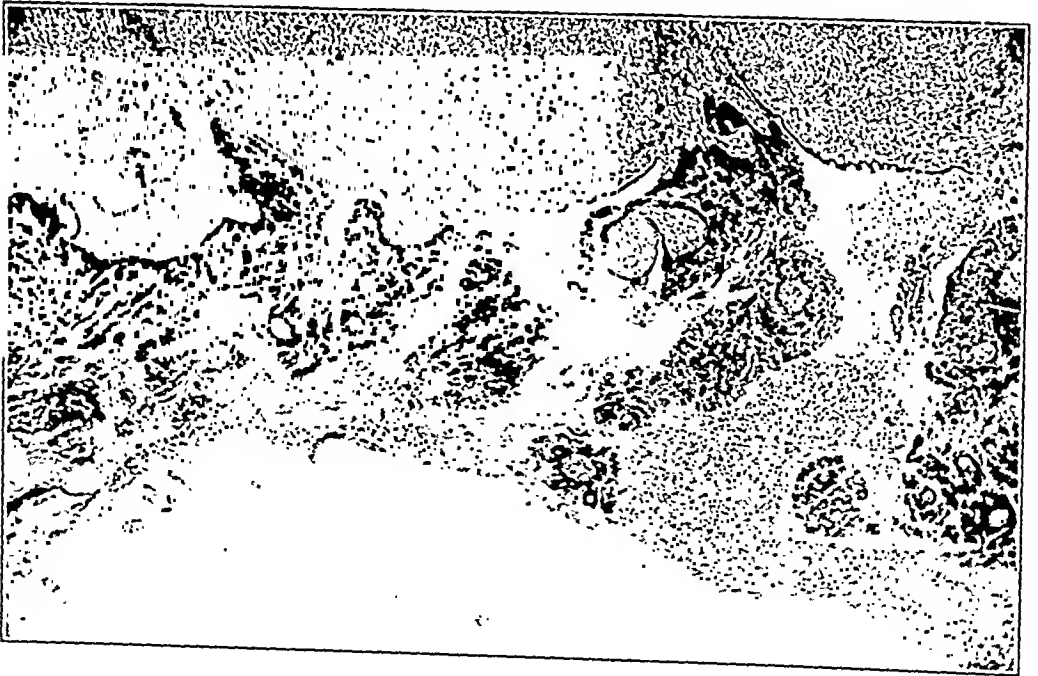
FIG. 11. Photomicrograph of a section through the posterior surface of the mesosalpinx (Case 4). The peritoneum, evidently, has been recently injured and shows a characteristic reaction. The mesothelium has been cast off from the peritoneum except in the center. Here fibrin has arched over the mesothelium, later to become organized and form a mesothelial inclusion. The surface of the denuded peritoneum is covered with fibrin. Clumps of epithelium-like cells are enmeshed in this fibrin. Were these cells derived from the cast-off mesothelium or from the cancer shown in Fig. 10? Histologically they are indistinguishable from the latter. The process, shown here, is identical with that of the fixation stage in the encapsulation of small foreign bodies experimentally introduced into the peritoneal cavities of the lower animals. Could it not also represent a similar stage in the implantation of cancer cells which we realize must have escaped into the peritoneal cavity?  $\times 130$ .

FIG. 12. Photomicrograph also through the posterior layer of the mesosalpinx but in a different location from that shown in Fig. 11. The entire surface of this portion of the peritoneum is denuded of mesothelium and covered by fibrin which has been infiltrated with cells growing in from the underlying tissue. The fibrin with its cellular contents has arched over a clump of epithelium-like cells deposited in a crevice of the mesosalpinx. This could readily represent the onset of the organizing stage in the implantation of cancer, similar to a like stage in the encapsulation of a foreign body, namely — the cellular infiltration of the fibrin covering a foreign body.  $\times 130$ .

FIG. 13. Photomicrograph of a section through the parietal peritoneum (Case 4). The surface of this portion of the peritoneum is denuded of mesothelium. The peritoneum is thickened and infiltrated with cells presenting the well known picture of irritation of long duration (compare with Figs. 11 and 12). Embedded in the thickened peritoneum are two clumps of epithelium-like cells identical in structure with those shown in the two preceding illustrations. It could well represent the organized stage in the implantation of cancer, identical with a similar stage in the healing of a peritoneal injury with the encapsulation of the foreign body causing the injury.  $\times 130$ .



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Implantation Peritoneal Carcinomatosis



PLATE 86

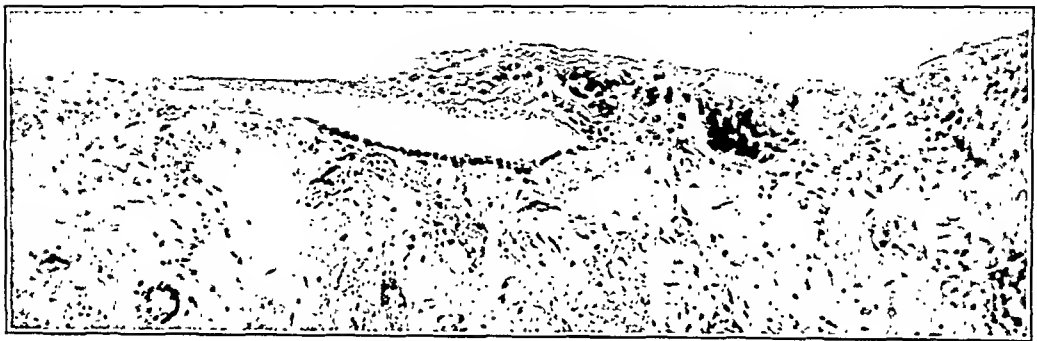
FIG. 14. Photomicrograph of a section through the posterior layer of the mesosalpinx (Case 4). Serial sections were made of the portions of the blocks shown in Figs. 11, 12, 13 and 14. The cancer in each instance was isolated and the slide photographed presented the greatest cross-section of the implant. The peritoneal surface here is denuded of mesothelium and covered by fibrin infiltrated with invading cells, a condition most favorable for the anchoring and encapsulation of a foreign body, the taking of a skin graft, or the implantation of cancer. Enmeshed in this fibrin is a relatively large mass of epithelium-like cells identical in structure with the malignant growth of the ovary (Fig. 10). Note that the activity of the fibroblasts is most marked just beneath the implant where the stimulation would likely be the greatest.  $\times 130$ .

FIG. 15. Photomicrograph of a later stage in the life history of cancer, anchored to the peritoneum by fibrin similar to the one shown in the preceding illustration. The growth is larger, the scaffold and temporary framework of fibrin has disappeared. Stroma has arisen from the fibroblastic invasion of the temporary framework. A like invasion by vascular endothelium has supplied blood vessels, thus forming granulation tissue. This represents the organizing stage in the development of an implant of polypoid type similar to a like stage in the repair of a wound containing a foreign body:  $\times 25$ .

FIG. 16. Photomicrograph of a section of the parietal peritoneum with two implants (Case 4). The larger one represents a later (the organized or healed) stage of the condition shown in the preceding illustration. The marked injection of the stroma of the implant and of the peritoneum shown in Fig. 15 has subsided. This implant is more definitely circumscribed and has settled into the underlying peritoneum which has been invaded by the cells of the implant, just as similar cells of a primary cancer invade underlying structures. The smaller implant (to the left) possibly might have arisen from conditions similar to those shown in Figs. 12 and 13. It is evident that the form of the implant varies with its age, the condition of the field of implantation when the cancer cells became anchored, the reaction of the tissues of its host and the rate of growth of the cancer.  $\times 25$ .



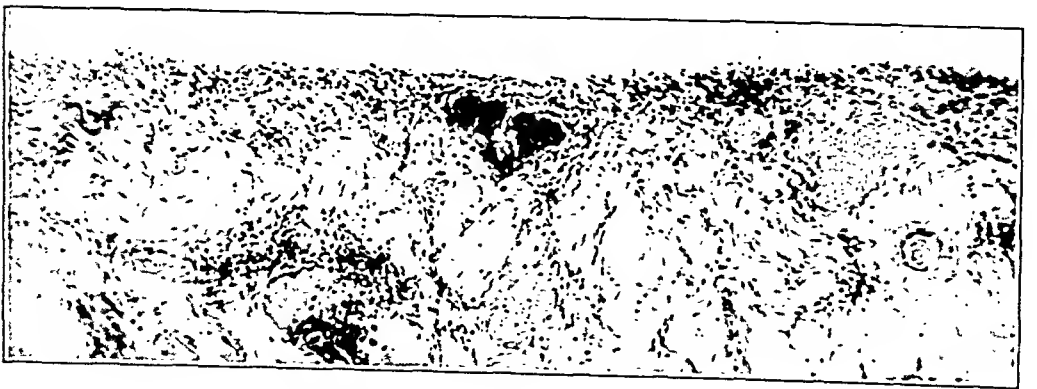
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## PLATE 87

FIG. 17. Photomicrograph of a cross-section of the Fallopian tube and a portion of its mesentery (Case 4). A polypoid implant is attached to the posterior surface of the mesosalpinx by a single slender vascular pedicle. The development of this type of implant is shown in Figs. 36 and 37. A tail-like process extends from the polypoid implant. It appears to be an outgrowth from the latter, but could well have arisen from the organization of a thin sheet of fibrin with enmeshed cancer cells. It is often of great importance to make serial sections of interesting lesions. Otherwise a single cross-section of a tail-like process similar to the one shown above may appear to be a small implant. This tail-like process was broad and flat, similar to a beaver's tail and not like that of a mouse as it appears in the photomicrograph.  $\times 10$ .

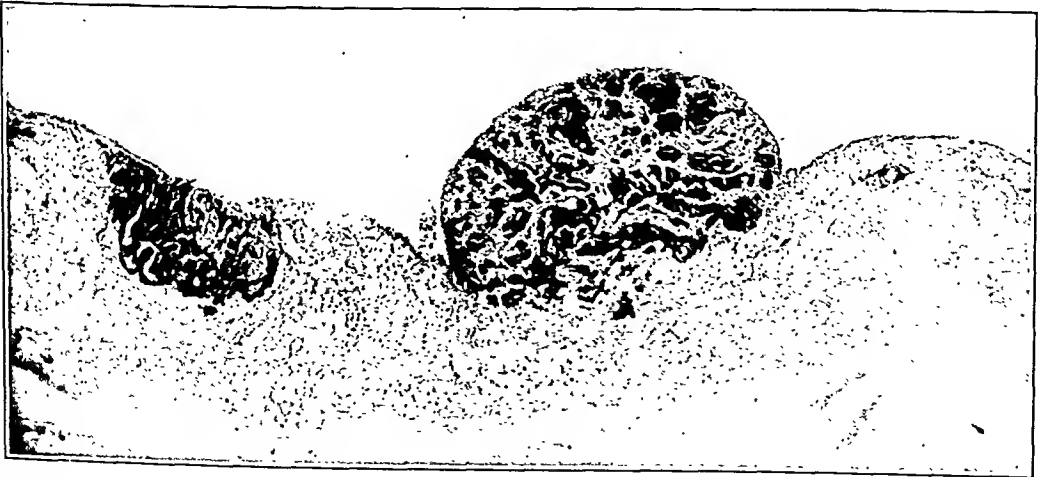
FIG. 18. Photomicrograph of a longitudinal section of an epiploical appendage (Case 4). The appendage is capped by cancer. The condition shown here well demonstrates how cancer, once implanted on the peritoneum, spreads over the surface of the latter, invades its underlying tissue (a) and when it pierces its capsule (b) then cancer cells might escape into the peritoneal cavity and be a source of cancer cells in the ascitic fluid; these in turn might become implanted on the peritoneum, causing secondary implants. The pedicle-like structure to the left arose from crushing the base of the appendage with a clamp when it was removed. Cancer was not found in the lymphatics of the appendage.  $\times 10$ .



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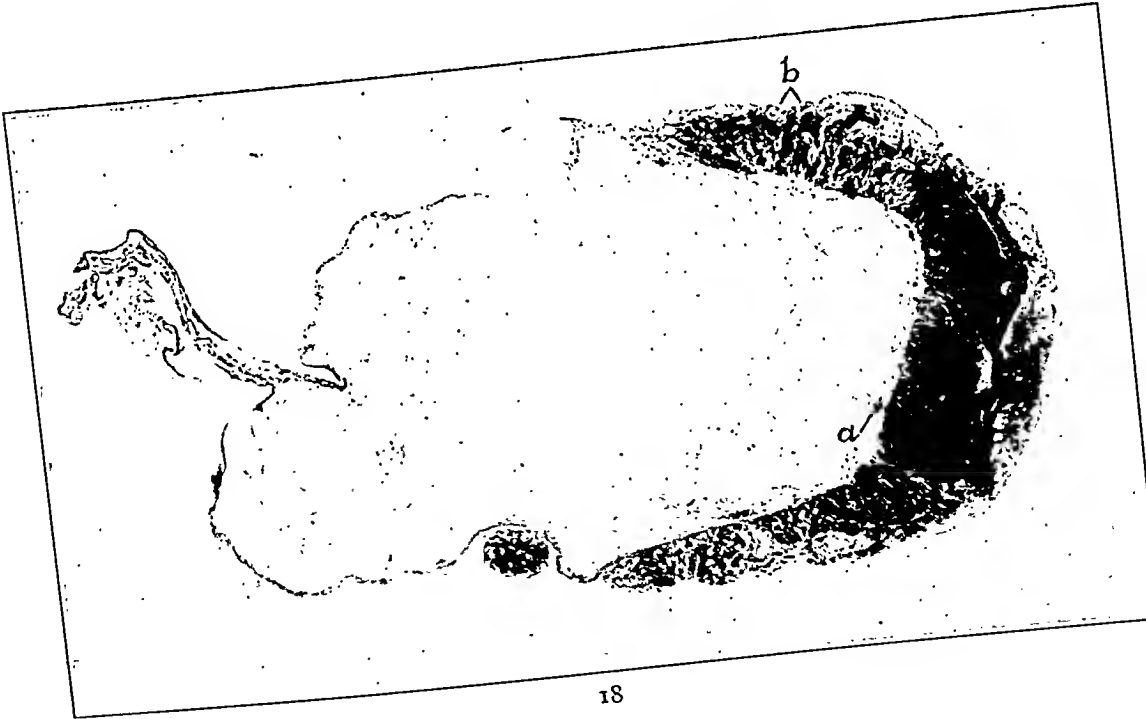
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## PLATE 88

FIG. 19. Photomicrograph of a cross-section of an obliterated appendix with a portion of its mesentery from a patient with peritoneal carcinomatosis associated with bilateral ovarian cancer (Case 5). Many interesting peritoneal lesions are present in this section. The wall of the appendix has been invaded on both sides at the attachment of its mesentery. This is a frequent site for implants on the intestinal tract. These two lesions are apparently of the same age, and as in many primary cancers, the oldest portion of the growth has sloughed, leaving a punched-out ulcer. An assumed earlier implant is present on the peritoneum of the mesentery ( (a) see also Fig. 22), and a still earlier one on the peritoneum of the appendix ( (b) see also Fig. 21). An encapsulated implant (c) is present at the apex of the illustration. Many of the cancer cells in this implant are dead. Cancer was not present in the lymphatics of the meso-appendix. For a probable source of the peritoneal lesions shown in this illustration see Fig. 20.  $\times 10$ .



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## PLATE 89

FIG. 20. Photomicrograph of a cross-section of the ovary with the tube and a portion of the meso-ovarium and mesosalpinx attached (Case 5). Almost the entire ovary has been replaced by cancer. The ovary was adherent by its lateral surface (lower border of the illustration) to the posterior layer of the broad ligament, invading the latter by direct extension. In other situations the cancer has extended through the capsule of the ovary, thus readily permitting the dissemination of cancer cells into the peritoneal cavity. The posterior layer of the mesosalpinx is invaded by cancer from its peritoneal surface. Similar but more superficial lesions are present on the anterior surface of the tube.  $\times 5$ .

FIG. 21. Photomicrograph of apparently an early implantation on the peritoneal surface of the appendix indicated by (b) of Fig. 19. As serial sections were not made of this block, one cannot exclude the probability that the condition shown here represents a section through the advancing edge of a larger implant over the surface of the peritoneum, rather than an early implantation. The peritoneum shows the characteristic lesion of chronic irritation; the hyperchromatic cancer cells are readily discernible. In one situation, to the right, they are surrounded by fibrin, but to the left they are partially surrounded by cells which I believe may be of mesothelial origin. I doubt if cancer cells can become implanted on intact mesothelium, but once established they might extend over the surface of the same or replace it.  $\times 130$ .

FIG. 22. Photomicrograph of the cancer involving the peritoneum of the meso-appendix, indicated by (a) of Fig. 19. Cancer embedded in the thickened peritoneum is shown at the right, and at the left, cancer enmeshed in fibrin infiltrated with cells growing out from the injured peritoneum. Serial sections were not made, and for this reason the exact relation between the cancer in the apparently different stages of its encapsulation cannot be determined. The cancer at the left could have arisen from the direct extension of that at the right rather than from a fresh implantation from the ovarian tumor.  $\times 60$ .

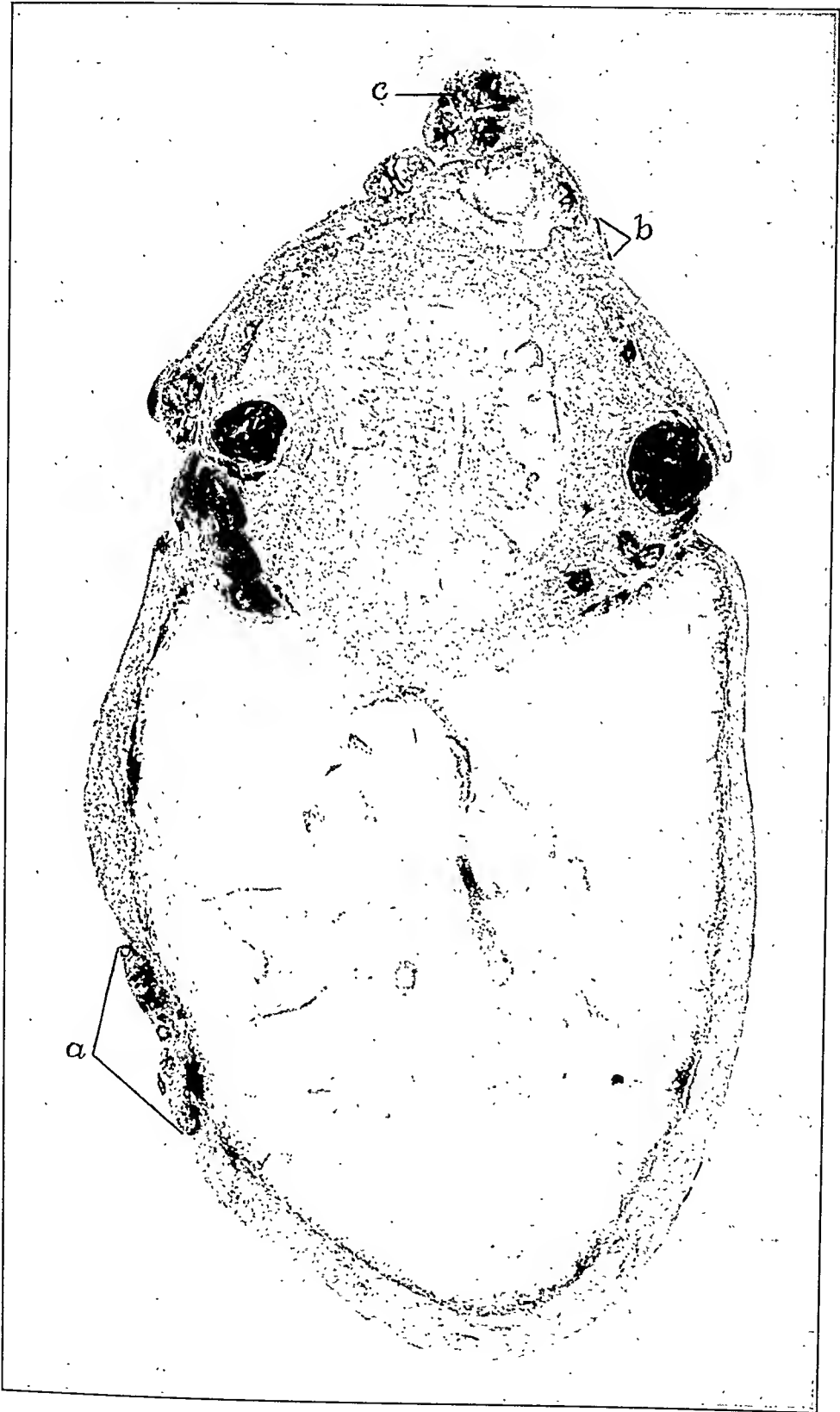
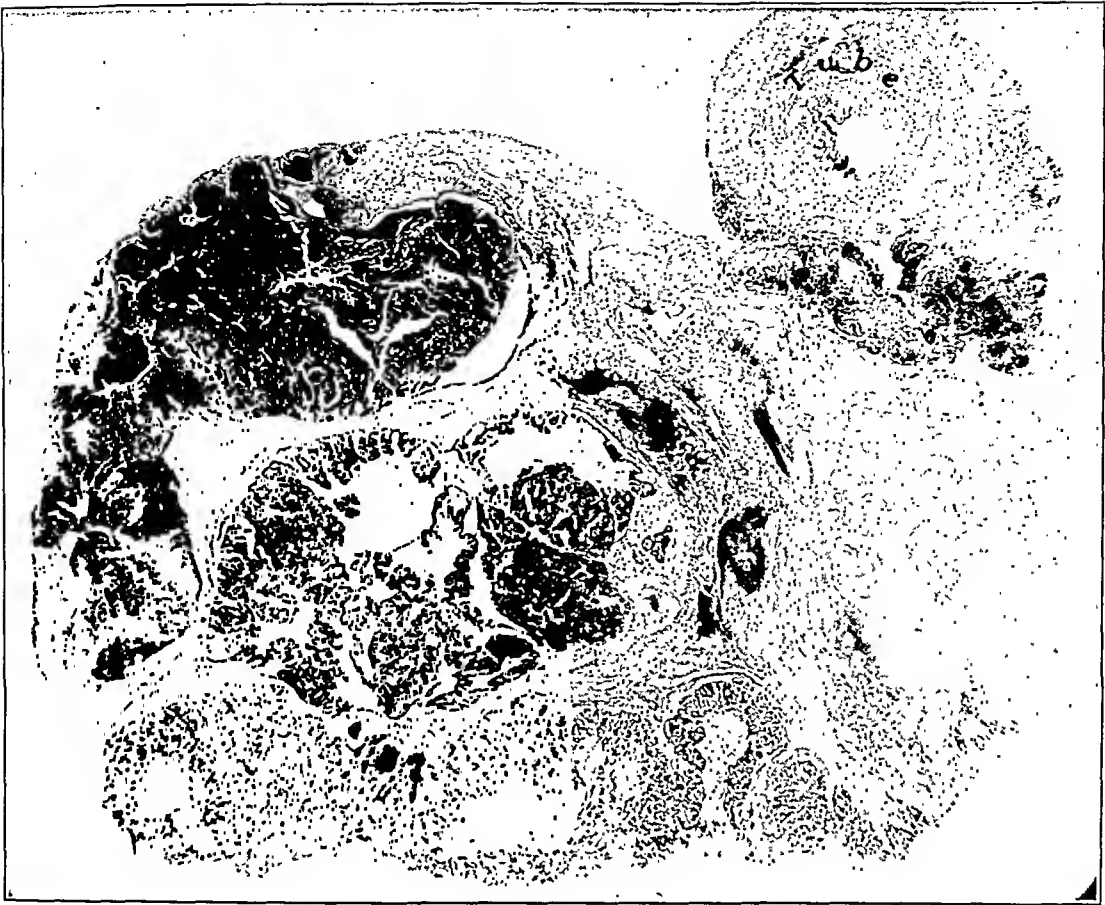




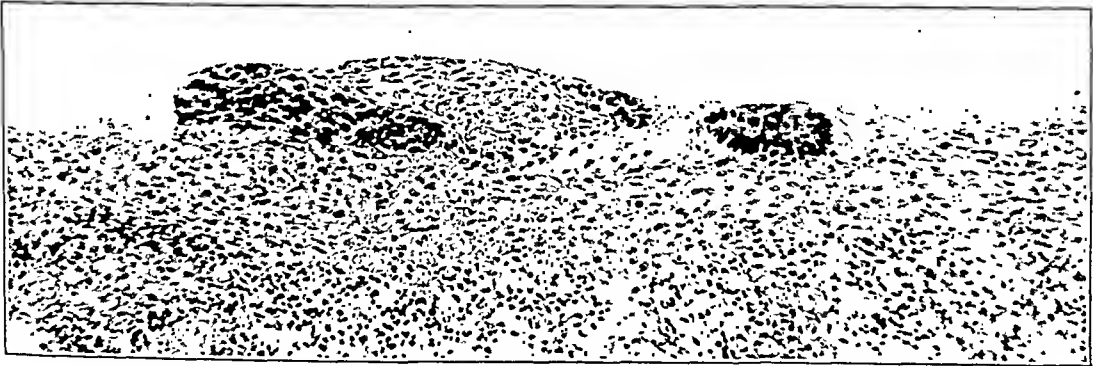
PLATE 90

FIG. 23. Photomicrograph of a cross-section of the tube with a portion of its mesentery (Case 5). Cancer was found only on the surface of the peritoneum: (a) is a large sessile, polypoid implant (granulation tissue stage) with a fenestrated base. Multiple pedicles have arisen from the organization of fibrin deposited on the surface of the peritoneum in an exudate which had poured out through multiple breaks in the mesothelial covering of the injured peritoneum. Through these same breaks fibroblasts and vascular endothelium grew out invading the fibrin. The fenestra are but the spaces between the pedicles. On the opposite side of the mesosalpinx are two older (organized) polypoid implants (b) representing the scar tissue stage in the healing of wounds caused by foreign bodies lodging on the peritoneum.  $\times 10$ .

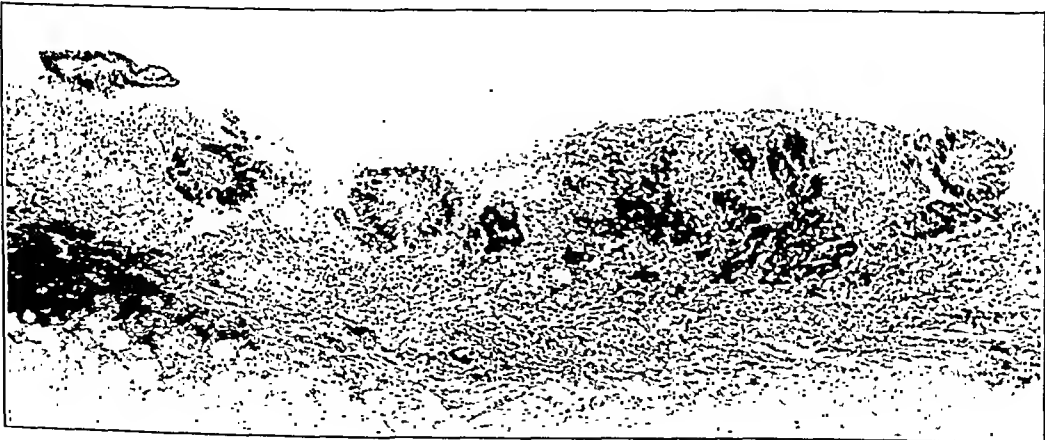
FIG. 24. Photomicrograph of a longitudinal section of an epiploical appendage (Case 5) showing various types of peritoneal implants, and various stages in their life history. At (a) the cancer is embedded in the thickened peritoneum like a foreign body. At (b) the implant is similar to the one shown at (a), but like a primary growth it has invaded the deeper tissues of the appendage (see Fig. 26). Implant (c) represents either an older implant or one more advanced than that shown at (a). It has invaded the tissues of the appendage en masse, as a primary cancer often does, and like the latter necrosis with sloughing and ulcer formation has occurred in the oldest part of the tumor. At (d) a papillary implant without encapsulation is spreading over the surface of the appendage (see Fig. 27). Cancer was not found in the lymphatics of the appendage.  $\times 10$ .



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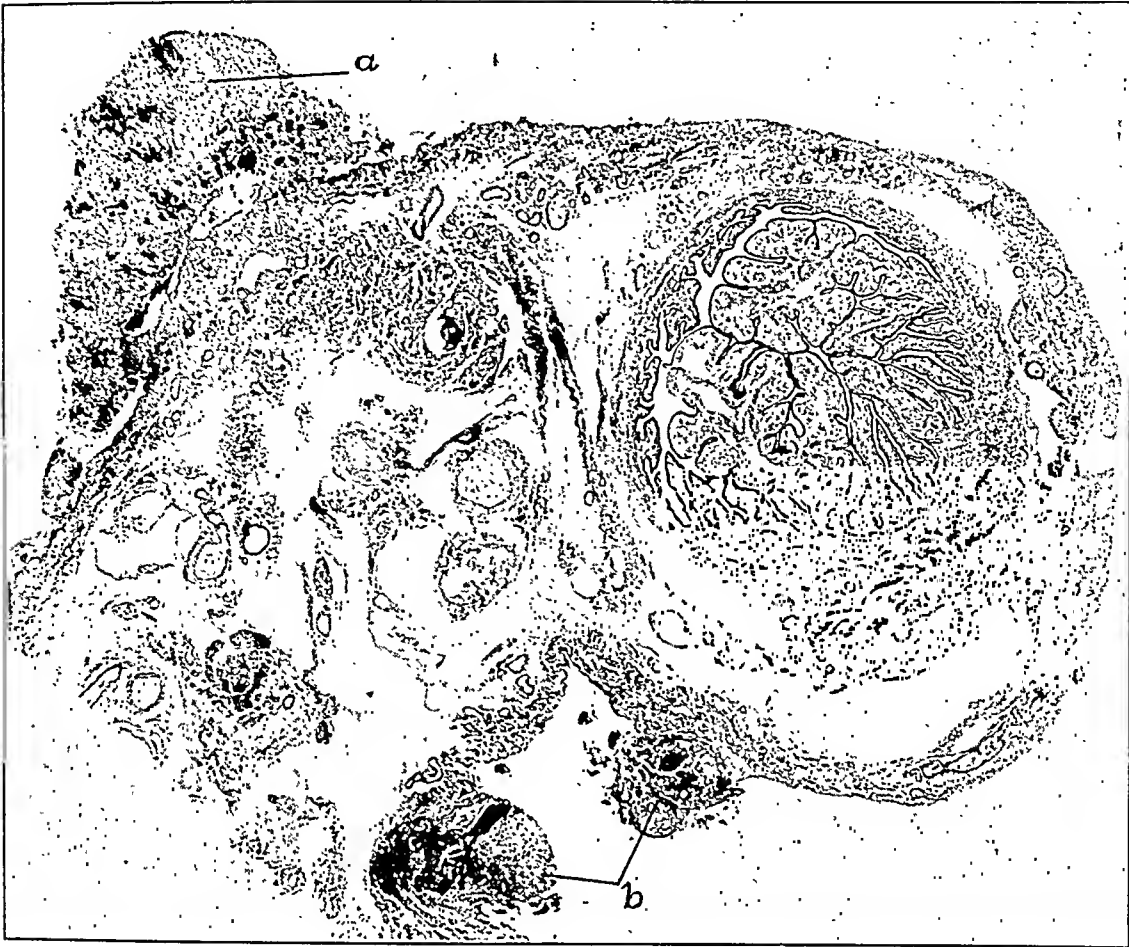
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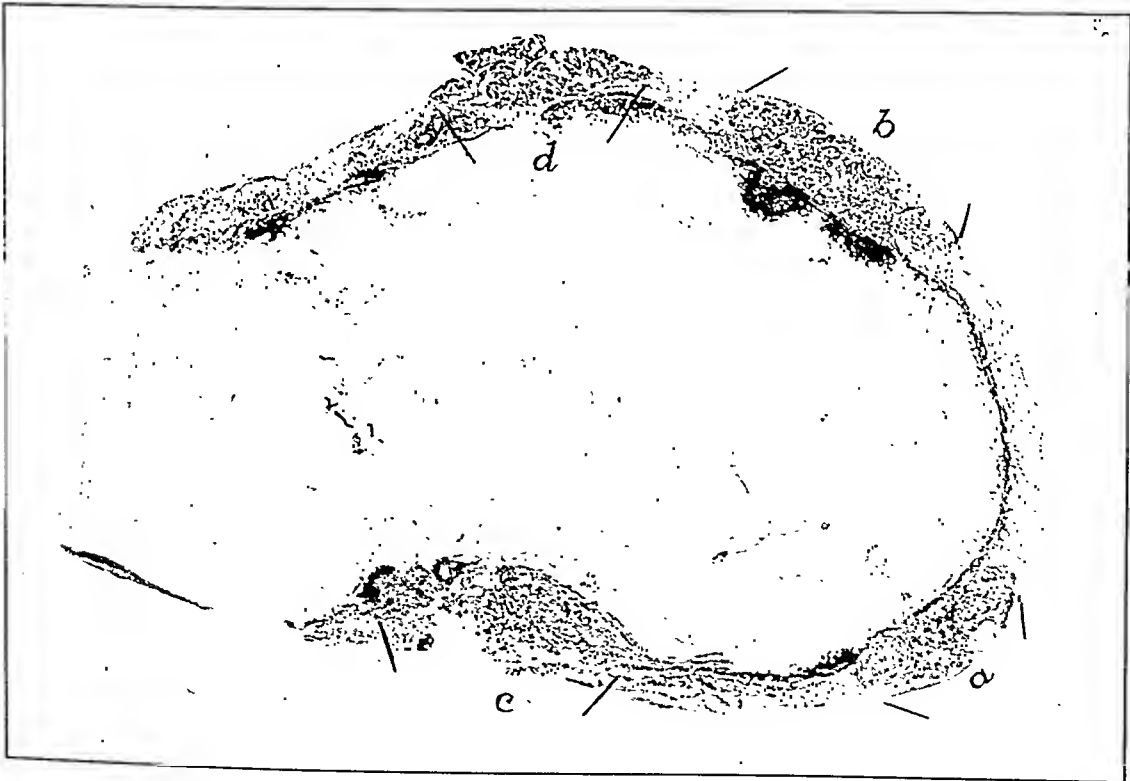
## PLATE 91

- FIG. 25. Photomicrograph of a portion of the peritoneum to the right of implant (c), Fig. 24. It well demonstrates the way newly formed tissue grows over cancer cells, later causing the condition shown in the next illustration. I am unable to state whether the clumps of cancer cells are a recent implantation, or a direct extension from the older implant. Serial sections were not made of this block.  $\times 60$ .
- FIG. 26. Photomicrograph of a portion of implant (b), Fig. 24. The implant is fully organized (the wound has been healed), but the cancer has invaded the subperitoneal tissues of the appendage like a primary tumor. Note the reaction to this invasion.  $\times 60$ .
- FIG. 27. Photomicrograph of a portion of implant (d), Fig. 24. It is a papillary tumor presenting the appearance of a primary cancer arising from the mesothelium of the peritoneum. I believe that it is an implant of the engrafted type without encapsulation, similar to the taking of a skin graft. Cancer cells were probably anchored to the peritoneum by fibrin and the fibrin covering the anchored cells never became organized, or if it did it was later destroyed by the cancer. Note the way the growth spreads over the surface of the appendage (to the right). Cells could escape from such a tumor into the peritoneal cavity as easily as from the primary tumor and might cause secondary peritoneal implantations.  $\times 60$ .



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Implantation Peritoneal Carcinomatosis

PLATE 92

FIG. 28. Cross-section of an ovary and tube from a patient with bilateral ovarian carcinoma, associated with ascites and a marked peritoneal reaction. This reaction manifested itself by an extensive distribution of granulation tissue on the surface of the peritoneum throughout the lower peritoneal cavity (Case 6). Cancer was not found in the lymphatics in any of the sections from this case. (Natural size.)

FIG. 29. Photomicrograph of a section through the exposed surface of the tumor shown in the preceding illustration. It is a papillary adenocarcinoma. A study of the conditions shown in this and the preceding illustration should impress one with two interesting probabilities — one, that cancer cells might easily escape from the surface of such a growth, and the other, that secretions, from the exposed cancer, escaping into the peritoneal cavity, as well as the cancer cells, possibly present in that fluid, might irritate (injure) the peritoneum.  $\times 60$ .

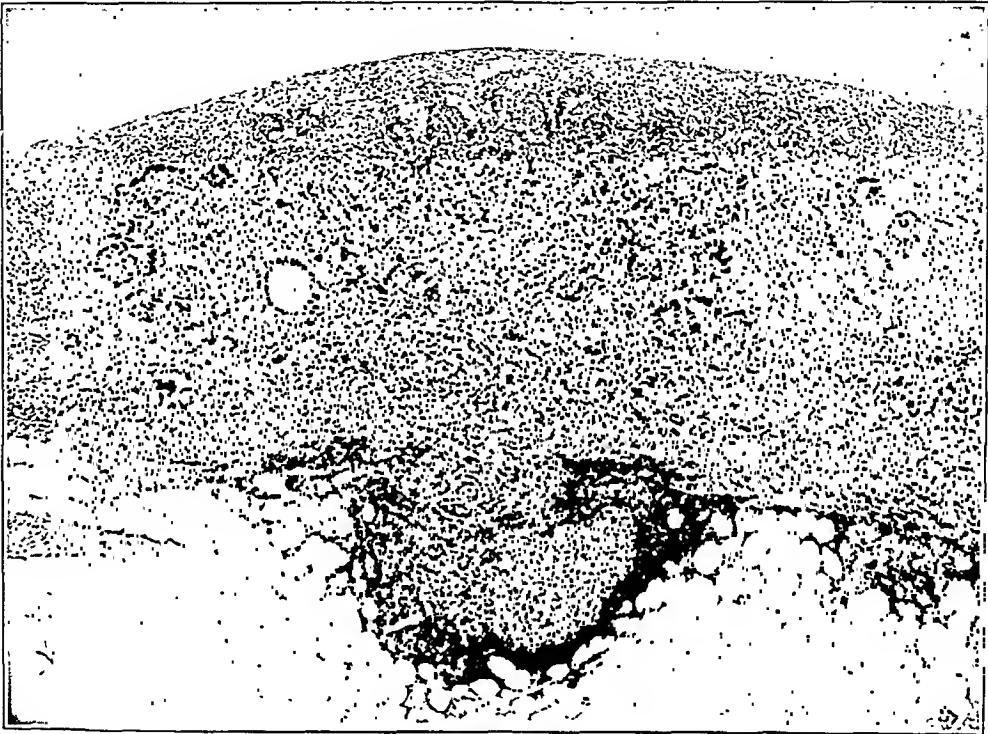
FIG. 30. Photomicrograph of a section of granulation tissue on the surface of an epiploical appendage (Case 6); see also Fig. 33. A clump of epithelium-like cells (c) is attached to the surface of a strand of fibrin. These cells both in appearance and arrangement are similar to those of the primary tumor shown in Fig. 29.  $\times 60$ .

FIG. 31. Photomicrograph of a section of granulation tissue on the surface of the appendix (Case 6). Epithelium-like cells (c) with an appearance and arrangement identical with similar cells in the two preceding illustrations are enmeshed in the granulation tissue. It might easily represent a later stage of that shown in the preceding illustration.  $\times 60$ .

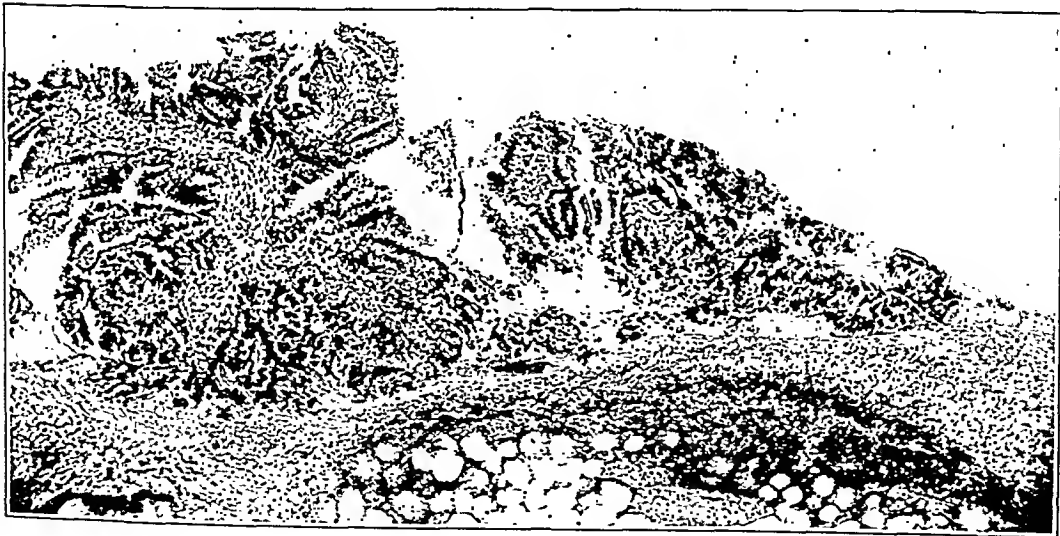
FIG. 32. Photomicrograph through granulation tissue on the surface of the mesosalpinx (Case 6). Epithelium-like cells with an appearance and arrangement identical with those in the three preceding sections are enmeshed in newly formed granulation tissue. On account of the proximity of the mesosalpinx to the primary tumor, these cells might have arisen from a direct extension of the primary growth, but this does not hold true for similar conditions in granulation tissue on the epiploical appendage and the appendix.  $\times 60$ .



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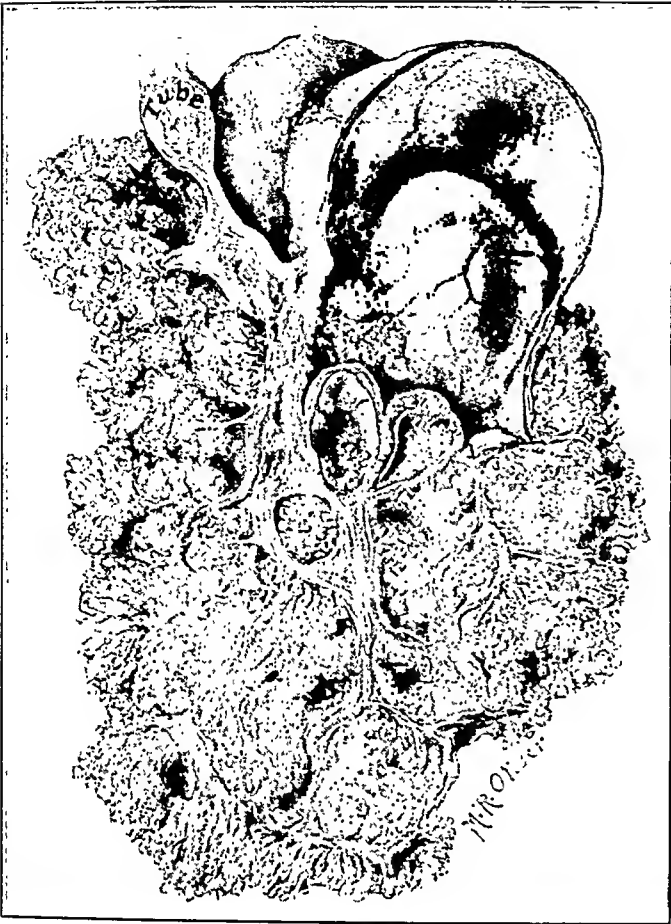
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## PLATE 93

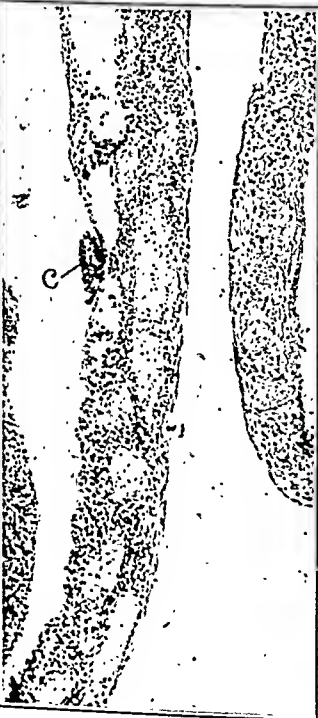
- FIG. 33. Photomicrograph of a cross-section of an epiploical appendage with granulation tissue on its peritoneal surface and mainly on one side of the appendage (Case 6). Note that the peritoneum covered with granulation tissue is much thicker than that without it. This suggests that the irritant causing the injury must have been applied more intimately to some portions of the peritoneum of the appendage than to others. As a result of the injury an exudate must have poured into the peritoneal cavity through breaks in the mesothelial covering of the appendage, leading to a deposit of fibrin on its surface. Through these same breaks in the covering of the appendage fibroblasts and vascular endothelium grew out, invaded the fibrin already described, and caused the granulation tissue shown above. The primary ovarian tumor (Figs. 28 and 29) suggests a source of the peritoneal irritant and the presence of cancer cells in this granulation tissue (see Fig. 30) taken at (c). Fig. 33 suggests that they are the irritant.  $\times 10$ .
- FIG. 34. Photomicrograph of granulation tissue on the peritoneal surface of an epiploical appendage from a patient with peritoneal carcinomatosis, associated with a malignant cyst of the left ovary. Clumps of cancer cells are enmeshed in the granulation tissue and also in fibrin just beneath it. The condition shown here could represent a little later stage of that shown in the preceding illustration, namely the organization of fibrin arising on the surface of the peritoneum as a reaction to some irritant which had escaped into the peritoneal cavity and was held captive in the organizing fibrin.  $\times 25$ .
- FIG. 35. Photomicrograph of a portion of the posterior wall of the uterus with fully organized adhesions on its surface in which cancer is embedded, from a patient with an extensive peritoneal carcinomatosis associated with a malignant cyst of the left ovary. The condition shown here represents a later stage of the one shown in the preceding illustration. The adhesions are fully organized, similar to the healed or scar tissue stage in the healing of a wound of the peritoneum caused by foreign bodies. Compare with Fig. 34 and note the marked growth of the cancer in the adhesions. The acute reaction of the former lesion has subsided, but the adhesions persist and the cancer continues to grow. Clumps of cancer cells are enmeshed in fibrin beneath the adhesions (to the left) probably representing a recent fixation of these cells on the peritoneum.  $\times 10$ .



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PLATE 94

FIG. 36. Photomicrograph of a longitudinal section of a polypoid implant which was attached to the posterior layer of the broad ligament by a slender vascular pedicle. The patient had peritoneal carcinomatosis associated with bilateral ovarian cancer. This represents the organizing or granulation tissue stage in the life history of this type of implant. The granulation tissue appears to have wrapped itself about the clumps of cancer cells. Probably the cancer cells were first caught in fibrin dangling into the peritoneal cavity, and this was invaded by fibroblasts and vascular endothelium, forming granulation tissue.  $\times 25$ .

FIG. 37. Photomicrograph of a longitudinal section of another polypoid implant with a slender pedicle, from the same patient as the one shown in the preceding illustration. The pedicle of this implant was attached to adhesions between the tube and ovary. It is a fully organized implant and may have resulted from a condition similar to the one shown in the preceding illustration. Compare with the condition shown in Fig. 36 and note the evident growth of the cancer.  $\times 25$ .

FIG. 38. Photomicrograph of a sessile polypoid implant on the posterior surface of the uterus. Like a primary growth it has invaded the uterine wall. It is from the same patient as the ones shown in the two preceding illustrations.  $\times 25$ .

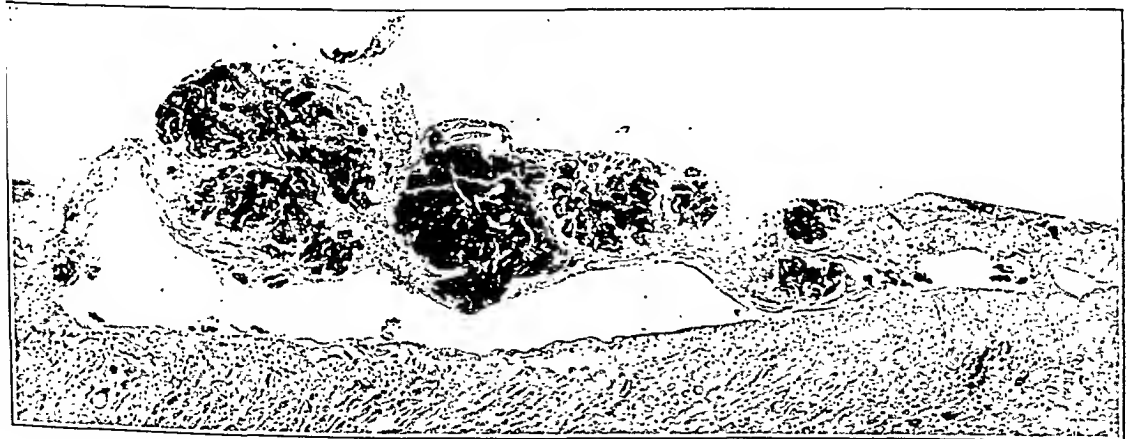
FIG. 39. Photomicrograph of two implants on the posterior surface of the uterus. Judging by the increased amount of scar tissue, they are older implants than the one shown in Fig. 38. All are from the same patient.  $\times 10$ .



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PLATE 95

FIG. 40. Pelvic contents from a patient with a peritoneal carcinomatosis associated with a cystic adenocarcinoma of the right ovary (Case 7). The peritoneal carcinomatosis was general, with the greatest involvement in the posterior cul-de-sac and omentum. In places, the cancer had extended through the capsule of the ovary and was thus exposed to the peritoneal cavity. (Natural size.)

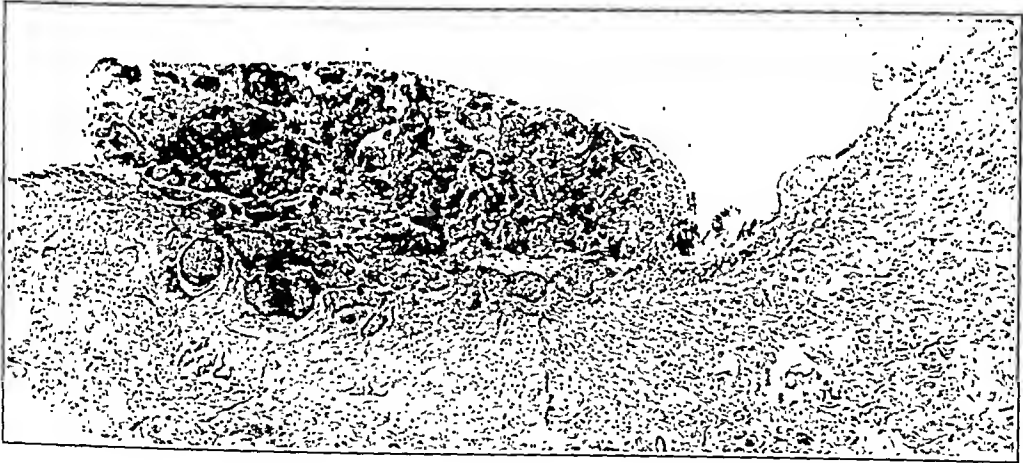
FIG. 41. Photomicrograph of a cross-section of the left ovary and tube and portion of the broad ligament (Case 7). Note that the latter is infiltrated with cancer and the ovary has been entirely replaced by the growth, identical in structure with that of the right ovary. The tube is free of cancer. The cancer in the left ovary was probably secondary to that in the right ovary. It had apparently invaded the ovary through its hilum. Is it from metastasis through the lymphatics or blood stream? Implantations on the posterior surface of the broad ligament, with invasion of the latter, and thence direct extension through the hilum of the ovary, could easily account for the condition shown in this photomicrograph. Cancer was not found in any of the retroperitoneal or mediastinal lymph nodes.  $\times 3$ .



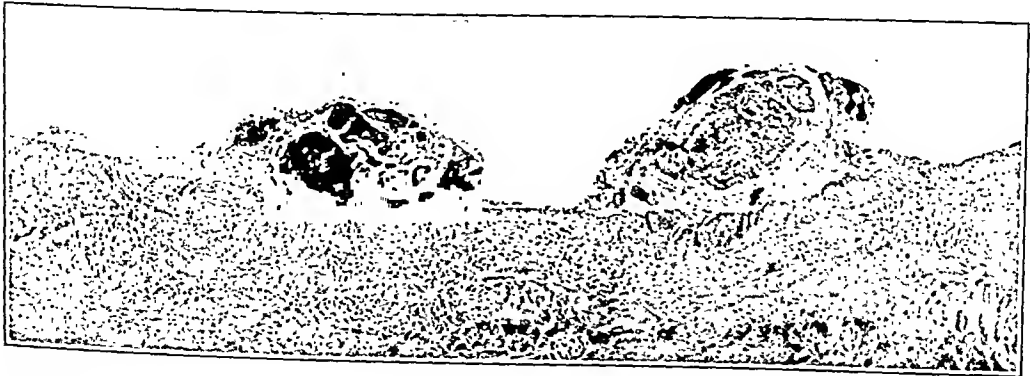
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Sampson

Implantation Peritoneal Carcinomatosis

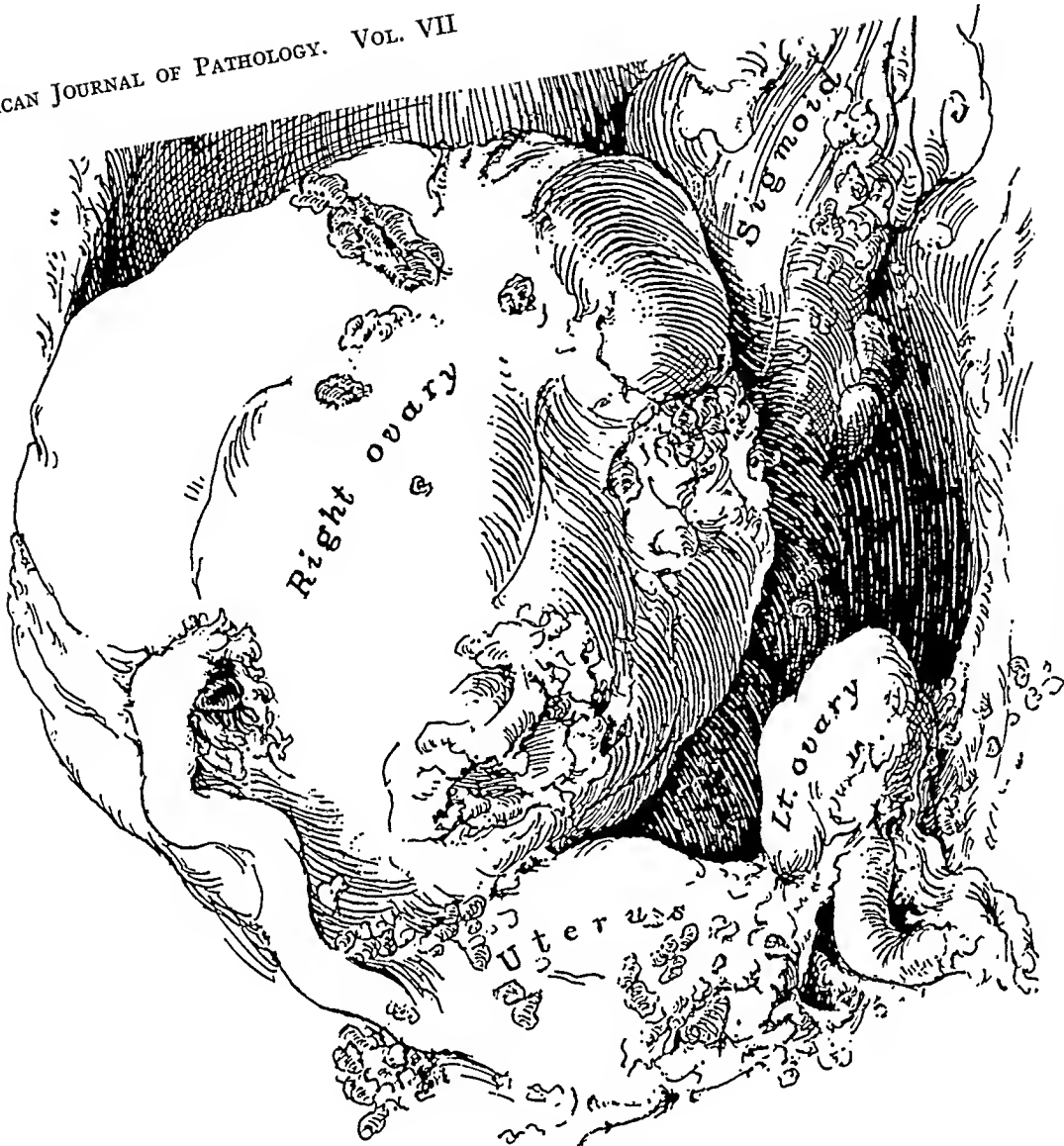
## PLATE 96

FIG. 42. Photomicrograph of an apparent metastasis on the under surface of the diaphragm (Case 7). Is it a true metastatic growth or did it arise from malignant changes in the mesothelium? I believe that it is probably an implantation of the skin graft type. The cancer cells became implanted without encapsulation. They were probably anchored with fibrin, but the latter was never organized by fibroblasts.  $\times 60$ .

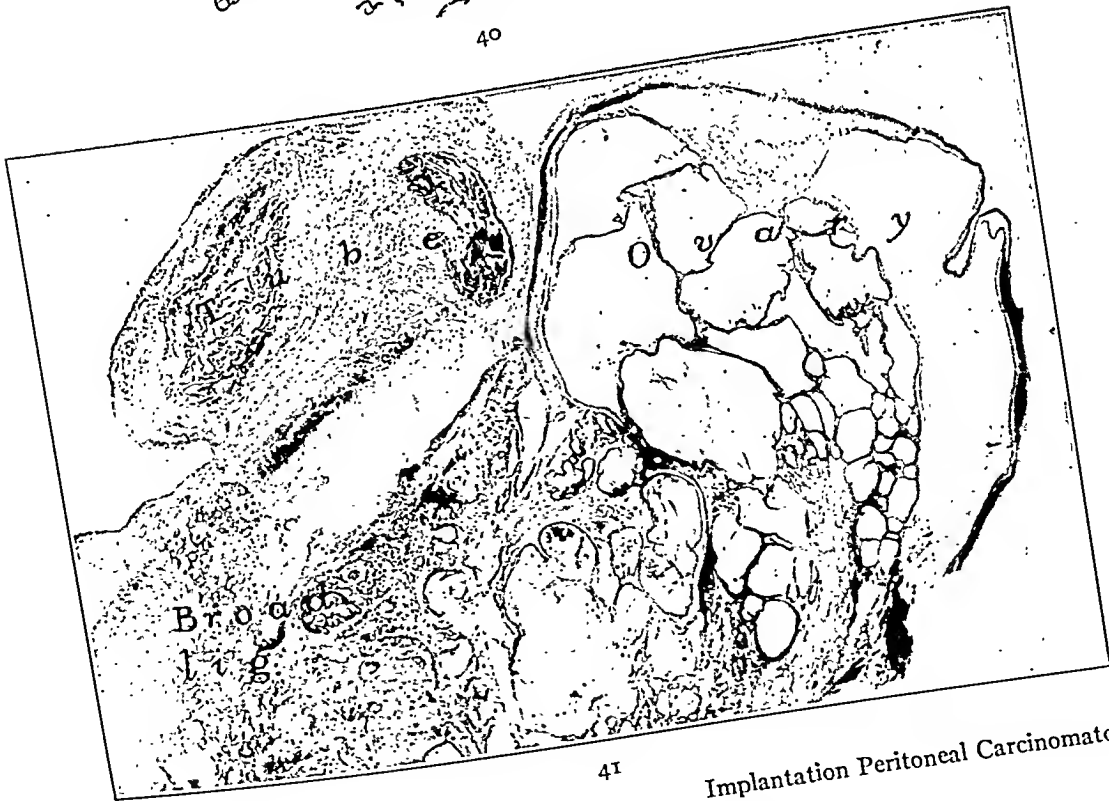
FIG. 43. Photomicrograph of an encapsulated implant on the diaphragm (Case 7). Cancer cells, in ascitic fluid (there was a large amount of it in this case), could easily reach the under surface of diaphragm on which the metastases shown in Figs. 42, 43, 44 and 45 were situated.  $\times 60$ .

FIG. 44. Photomicrograph of another implant on the diaphragm. It is apparently an older implant than the preceding one. It is broader, flatter and there is more connective tissue.  $\times 60$ .

FIG. 45. Photomicrograph of still another implant on the diaphragm, which I believe is older than the preceding one. It is flatter, larger and there is a deeper invasion of the underlying tissues and a more extensive invasion of its capsule. Cancer is present on the surface of the implant at (a). Does it represent a fresh implantation of cancer in this situation or an extension of the cancer through the capsule to its surface? Serial sections were not made of this metastasis and therefore this point cannot be determined.  $\times 25$ .



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Implantation Peritoneal Carcinomatosis

PLATE 97

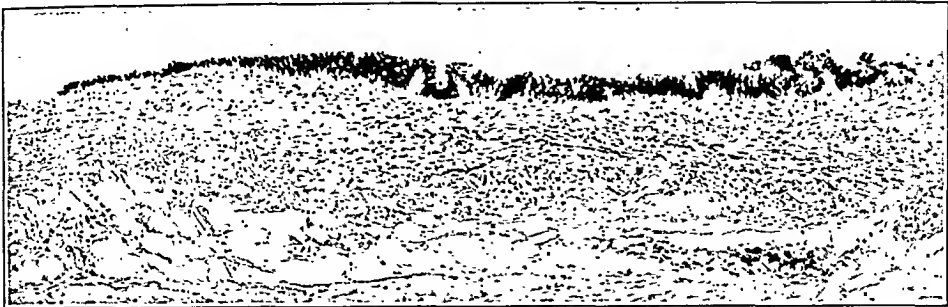
FIG. 46. Photomicrograph of a section of a portion of an epiploical appendage showing two implants of papillary type — the larger one is partially encapsulated — from a patient with extensive peritoneal carcinomatosis associated with bilateral ovarian cancer. An encapsulated implant from the same patient is shown in Fig. 48.  $\times 25$ .

Fig. 47. Photomicrograph of a section of a portion of an epiploical appendage from a patient with a condition similar to the preceding one. The patients were sisters. Note the similarity in the two tumors. The tumors shown in Figs. 46 and 47 have the appearance of primary growths arising from the mesothelium of the appendage. I believe that they are metastatic by implantation from the ovarian tumors or from other implants. Implantation may have occurred without encapsulation, just as the epithelium of a Thiersch skin graft takes. Even if primarily covered by fibrin this may not have become organized and therefore disappeared. It is also possible that encapsulated cancer may destroy the capsule over it. A study of the condition shown in these two photomicrographs emphasizes the ease with which cancer cells could escape from these tumors into the peritoneal cavity and give rise to secondary implants.  $\times 25$ .

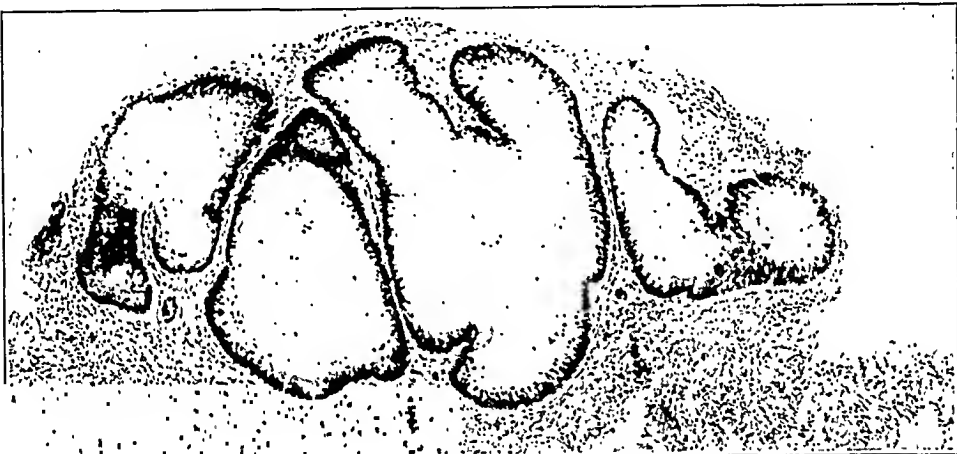
FIG. 48. Photomicrograph of cancer embedded in the peritoneum of the anterior cul-de-sac near the uterus, from the same case as the epiploical appendage shown in Fig. 46. I believe that the condition shown here may have arisen from implantation on the peritoneum with encapsulation and subsequent growth of the cancer, rather than from cancer cells carried through the lymph or blood vessels.  $\times 10$ .

FIG. 49. Photomicrograph of a section of sediment of ascitic fluid obtained from the same patient as the implant shown in Fig. 47, six months after a previous operation. The mass of pale cells I believe is of mesothelial origin. The clumps of hyperchromatic cells with a glandular arrangement could well be cancer cells which had escaped into the peritoneal cavity from implants such as the one shown in Fig. 47, or they may have been present in the fluid at the first operation when the uterus, tubes and ovaries were removed.  $\times 60$ .

FIG. 50. Photomicrograph of a small peritoneal implant removed at the second operation. It is evidently an old implant probably present at the first operation.  $\times 60$ .



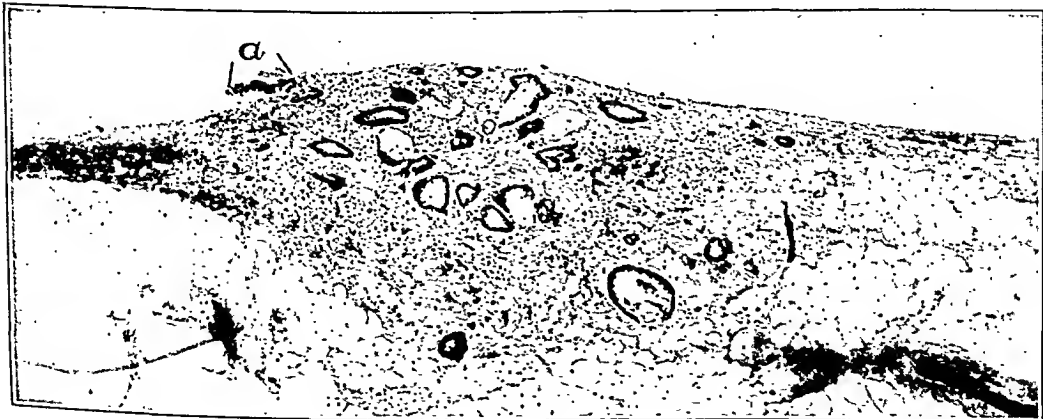
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## PLATE 98

FIG. 51. Photomicrograph of a small implant on the parietal peritoneum (Case 4). Compare with Fig. 16 from the same patient. It appears older than the larger one shown in Fig. 16. It is flatter, more definitely encapsulated, and is more deeply sunken in the peritoneal tissues. Fine root-like processes of cancer from the base of the implant have invaded the deeper tissues of the peritoneum. Cancer is present in a lymphatic at (c). Every metastatic tumor has the same potentialities of invasion and dissemination as a primary tumor in a similar situation. Some may claim that the cancer in a lymphatic indicates that these are the channels by which cancer cells had escaped from the primary tumor to this situation.  $\times 25$ .

FIG. 52. Photomicrograph of an implant in an epiploical appendage (Case 2, see also Figs. 3 and 4). Note the invasion of the epiploical appendage and the reaction of the tissues of the appendage to this invasion. It is possible that in time the entire metastasis might have been fully embedded in the appendage. Had the section been at right angles to the one shown here, the cancer might have appeared to be in the center of the appendage.  $\times 25$ .

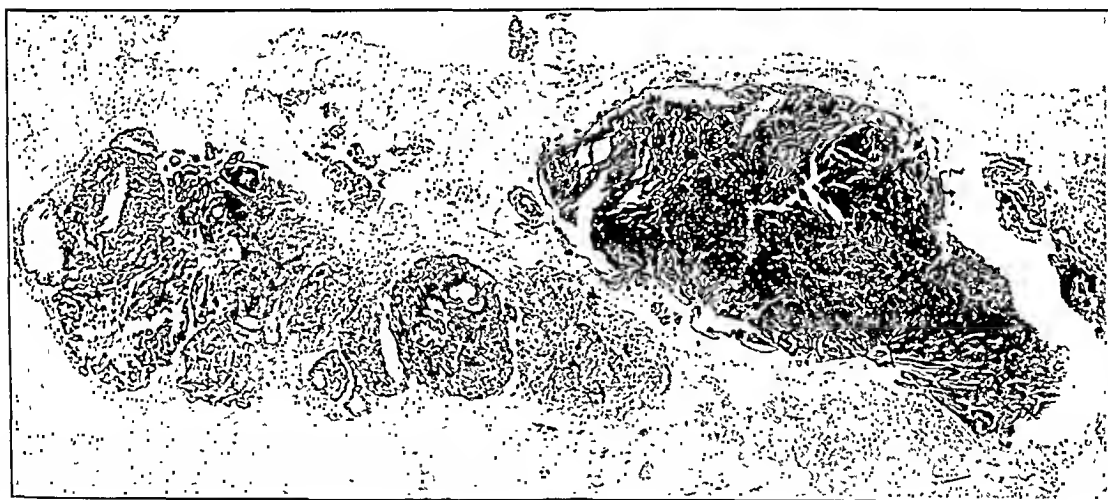
FIG. 53. Photomicrograph of a cross-section of an epiploical appendage from a patient with extensive peritoneal carcinomatosis associated with bilateral ovarian carcinoma. I believe that the deeper tissues of the appendage could well have been invaded by cancer implanted on its peritoneal surface. Others might claim that the peritoneal involvement arose from lymphatic metastases from the primary tumor, and the cancer in the deeper tissues of the appendage are an indication of this.  $\times 10$ .



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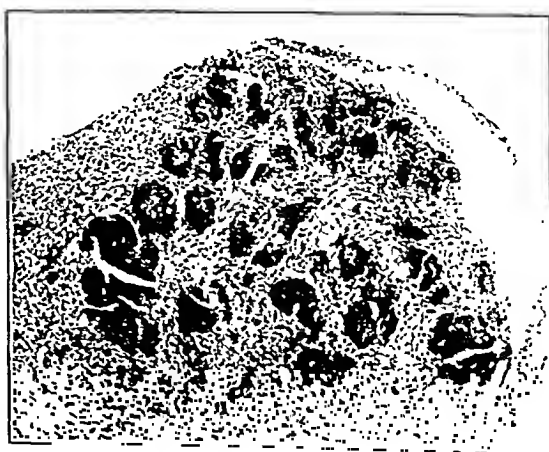
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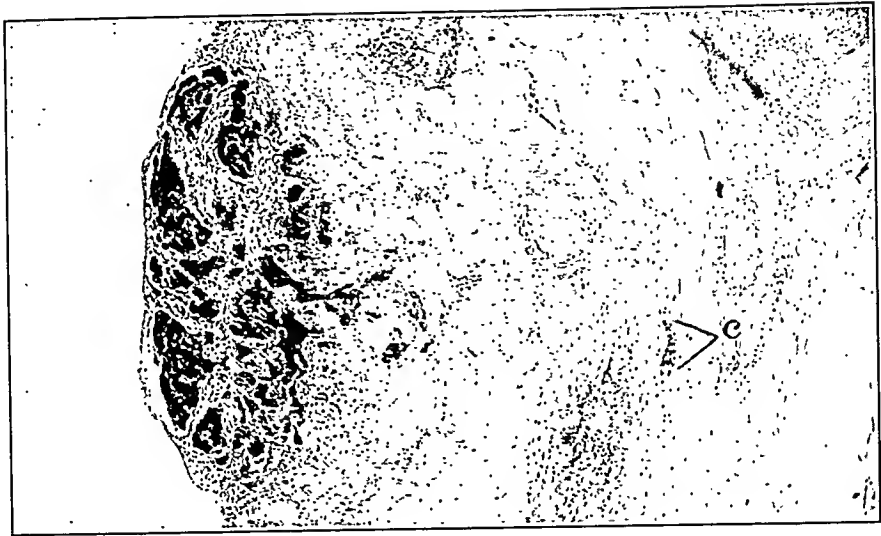
Sampson

Implantation Peritoneal Carcinomatosis

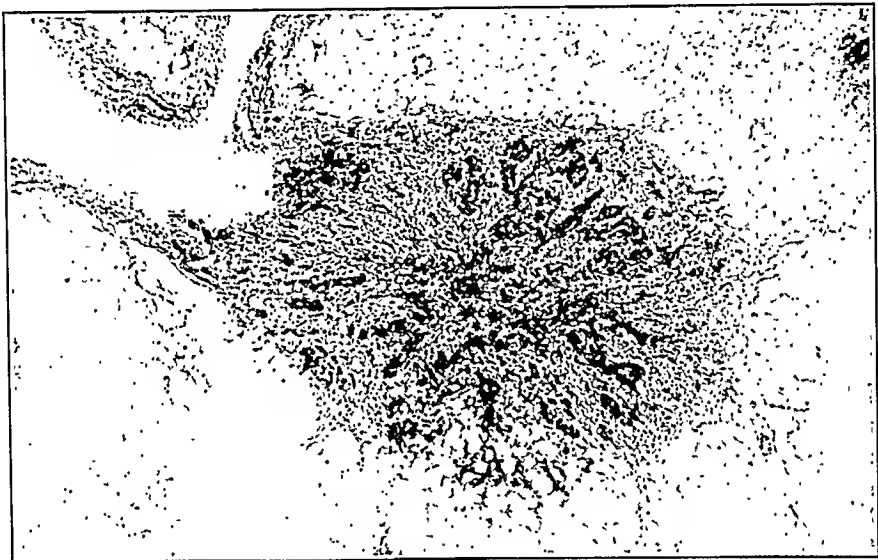
PLATE 99

FIG. 54. Photomicrograph of a cross-section of an appendix with a portion of its mesentery, from a patient with extensive peritoneal carcinomatosis associated with bilateral carcinomatous ovarian cysts. Two fully organized implants are shown, one on each side of the appendix at its mesenteric attachment, a frequent site for implantations on the intestinal tract. They are apparently of about the same age, at least they represent the same stage in their development. Compare with Fig. 34 in which is shown an implant in adhesions on the epiploical appendage from the same patient, the latter representing the organizing or granulation tissue stage in the development of peritoneal implants. The cancer in both of the implants shown here has invaded the deeper tissues of its host like that of a primary growth. (See next illustration.)  $\times 10$ .

FIG. 55. Photomicrograph of a portion of implant (a), Fig. 54, and the wall of the appendix. The cancer cells of the implant have invaded the wall of the appendix as those of a primary growth might. Cross-sections of vessels (probably lymphatics) containing cancer cells are shown at (c) and (c). Did the latter arise from the cancer of the implant or from the ovarian tumor? I believe the former. Cancer was not found in the lymph vessels of the meso-appendix. What might be a later stage of the condition shown in these two photomicrographs?  $\times 60$ .



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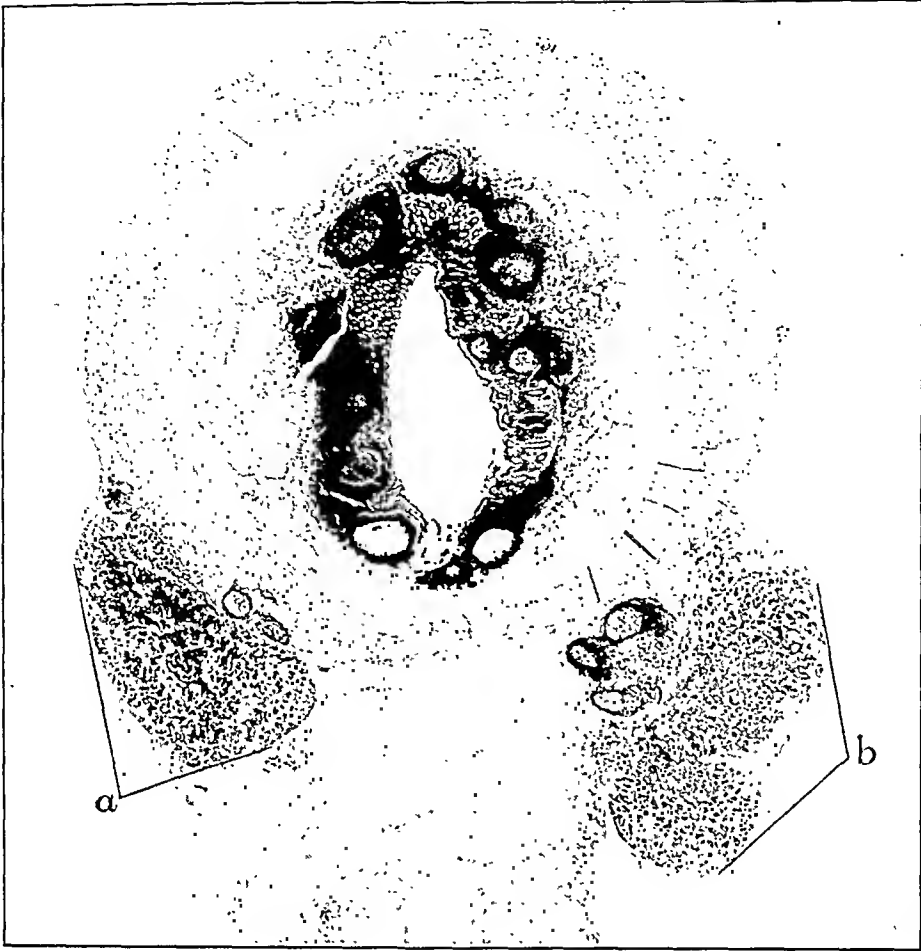
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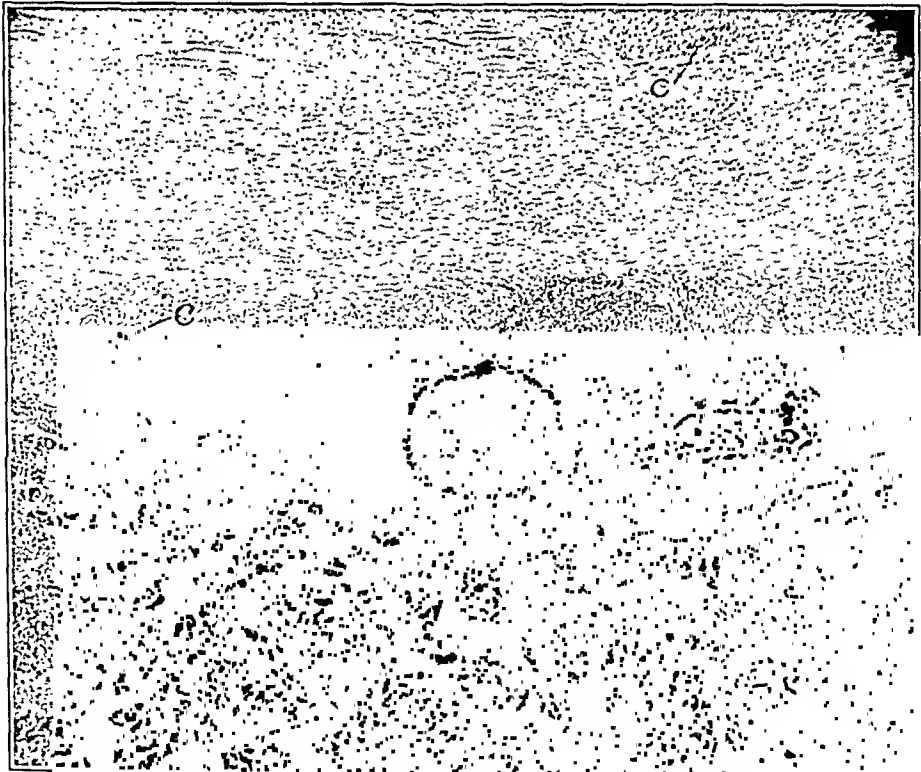
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## PLATE 100

- FIG. 56. Photomicrograph of a cross-section of an appendix with a portion of its mesentery, from a patient with peritoneal carcinomatosis associated with a carcinomatous cyst of the left ovary. The peritoneal metastasis (implant) is evidently older than those shown in Fig. 54. It is flatter and more intimately blended with the wall of the appendix and the tissues of its mesentery. The lumen of the appendix is obliterated and the lymphatics of the appendix, as well as those of the mesentery, are permeated with cancer filling them like an injection mass. See Figs. 58 and 59.  $\times 10$ .
- FIG. 57. Photomicrograph showing cancer cells enmeshed in fibrin and partially embedded in granulation tissue from (a) of the preceding illustration. As serial sections were not made of this block it is impossible to state whether this is a recent implantation or a direct extension from the cancer to the left. From a histological standpoint it could be either.  $\times 60$ .
- FIG. 58. Photomicrograph showing the extension of cancer from the base of the implant into the tissues of the wall of the appendix ((b) Fig. 56). Lymph vessels permeated with cancer are shown just beneath this invasion.  $\times 60$ .
- FIG. 59. Photomicrograph showing a portion of the appendix and its mesentery ((c) Fig. 56). There is a gap in the muscularis of the appendix for the entry and exit of vessels. The lymph vessels of both the appendix and its mesentery are filled with cancer cells. It might be claimed that the conditions shown in these photomicrographs indicate that the metastatic cancer of the peritoneum of the appendix came from the ovarian tumor through the lymph vessels. The metastatic growth of the peritoneum presents the histological appearance of an older implant than the one shown in Fig. 54, and therefore with greater opportunities for invasion and lymphatic permeation. I believe it could well represent a later stage of the former. The conditions found in both implants are frequently seen in primary cancers and a metastatic tumor has the same potentialities of invasion and dissemination as a similar primary growth in its situation.  $\times 60$ .



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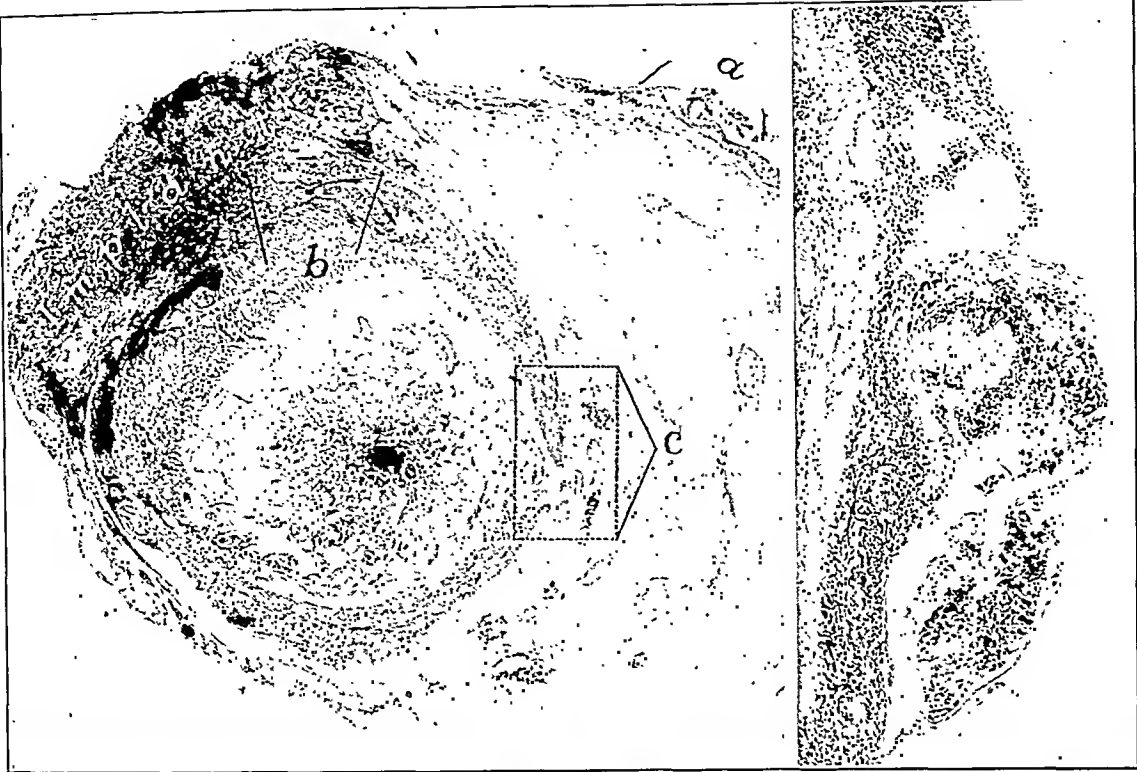
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PLATE 101

FIG. 60. Colored photomicrograph of the section shown in Fig. 11 (Case 4) to demonstrate better the reaction of the peritoneum to injury caused by cancer cells (c) lodging on its surface. Note the injection of the blood vessels and the other reactions described in Fig. 11. I believe that the condition shown here represents the fixation stage in the life history of the implantation of cancer cells on the peritoneum.  $\times 130$ .

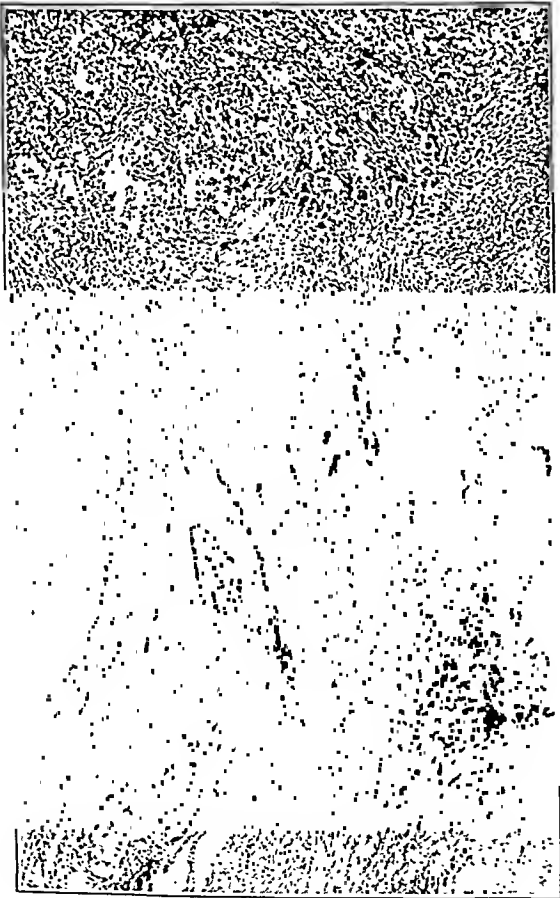
FIG. 61. Colored photomicrograph of a section of a peritoneal implant on the surface of the Fallopian tube shown in Fig. 20. It represents the organizing stage in the life history of such an implant. The clumps of cancer cells are enmeshed in granulation tissue, a well recognized reaction to peritoneal injury. This undoubtedly arose from the invasion of fibrin, on the surface of the injured peritoneum, by fibroblasts and vascular endothelium growing out through the break in the peritoneal mesothelium shown in the center of the illustration. Compare with Fig. 60 and note the changes in the peritoneum, indicating an irritation of longer duration than that shown in that illustration.  $\times 60$ .

FIG. 62. Colored photomicrograph of a section through an implant on the posterior surface of the mesosalpinx (Case 4). It is a fully organized implant of the polypoid type. Note the evident growth of the cancer in the implant, the attempt at encapsulation, and the extension of the cancer through the capsule in places. An apparent contact peritoneal implant from the larger implant, above it, is situated at (a) and an implant similar to the one shown in Fig. 60 is situated at (b). The latter also might have arisen from cancer cells escaping from the larger implant. The larger implant had two vascular pedicles, portions of which are shown at (p) and (p).  $\times 25$ .



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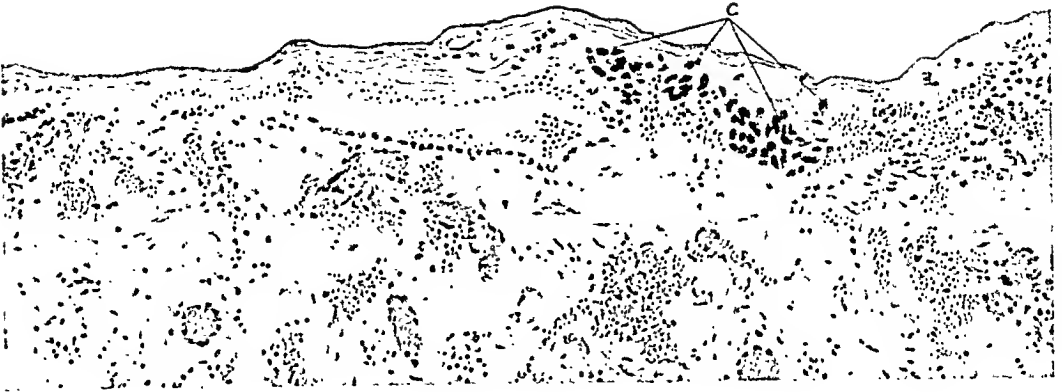
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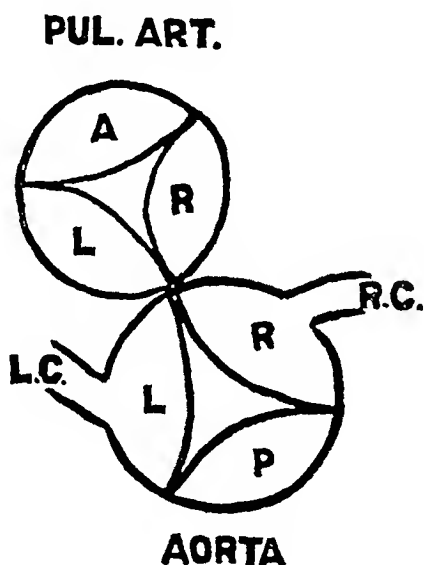


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In this paper we propose to describe and picture the valves of the human heart, both in their more minute histology as well as in their typographical relations to other structures, setting as far as possible the limit as to what is valve and where the adjoining cardiac structures begin. We shall also attempt to establish a descriptive nomenclature and indicate at the same time what changes occur in the various structures at different age periods. In our experience with pathological anatomy we feel that the latter point, to which practically no attention has been paid, may carry with it some leads on the mechanical factors concerned with valvular disease.



TEXT-FIG. 1. Diagram to illustrate terminology of pulmonary and aortic valve cusps.  
 LC, left coronary artery; RC, right coronary artery; L, left cusp; R, right cusp; A, anterior cusp; P, posterior cusp.

The plan is to discuss the general structure of valves in brief and to follow this by a detailed description of each cusp, instead of grouping together the semilunar valves and the auriculoventricular valves, as has been the general practice heretofore.

In this article the B. N. A. nomenclature is used (Text-Fig. 1). Thus, the aortic cusp corresponding to the ostium of the right coronary artery is called the right cusp, that corresponding to the left coronary ostium is called the left cusp and the non-coronary cusp is called the posterior. The right pulmonary cusp is that one which is apposed to the right aortic cusp. The left pulmonary cusp is that

## TOPOGRAPHIC ANATOMY AND HISTOLOGY OF THE VALVES IN THE HUMAN HEART \*

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An accurate knowledge of the structure of the valves in the human heart is of prime importance for at least three very practical reasons. The first is that the various anatomical peculiarities in the valve leaflets suggest an explanation for at least some of the mechanical components entering into the localization of inflammatory, as well as of degenerative (atherosclerotic) processes in these sites. Secondly, the insight into pathological processes thus obtained helps one differentiate these lesions from one another. Thirdly, one is better able to cope with the long disputed question as to whether or not blood vessels or myocardium normally exist in valves. This is not a merely academic question, myocardium having been implicated by various authors as the source of the alleged existence of blood vessels in normal valves.

The descriptions of the valves found in the literature are difficult to follow because the best ones are given by workers who limit themselves to only one or two valves and, since the terminology used is often different, confusion results. As an illustration of this, one may mention that the same layer of tissue in the valve is called "Klappenplatte" by Seipp, "Mittelschicht" by Beitzke, "Grundstock" by Königer, "Lamina Fibrosa" by Tandler, "Klappenskelet" by Benninghoff, and by other investigators is referred to by number. Perhaps the greatest source of dispute lies in the fact that, with rare exceptions, no attempt is made to set definite limits to the valve. It may be said at once that this factor alone is largely responsible for the controversy on the extent to which myocardium has been found in valves and, because of this, on the frequency with which blood vessels have been found in the valves.

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† Moses Heineman Fellow in Pathology.

differs considerably with the various cusps in its extent, distribution and connections. Its topography for the aortic valve has been admirably described by Lewis and Grant. There exists, however, an important strategic site which includes part of the base of the valve as well as the adjacent portion of the *Annulus*. We designate this area the *Ring* (Text-Fig. 2). The exact descriptions of the several valve *Rings* will be given under their appropriate headings. *It is of utmost importance to mention here that the valve "Ring" should be considered to constitute the proximal end of a valve leaflet.* This will be taken up further in greater detail.

The *Rings* of the semilunar valves (Fig. 5 and Text-Fig. 2) generally contain a conspicuous *Spongiosa* referred to by Curtis as "un espace triangulaire . . . occupé par un tissue conjonctif spécial." On the other hand, the auriculoventricular valve *Rings* in this area show only a slightly looser structure of the collagenous *Fibrosa* (Fig. 8). We designate these areas *Ring Spongiosa*.

The *Fibrosa*, with its looser layer, is clothed on both sides by a continuation of the arterial intima or ventricular or auricular endocardium, as the case may be. These arterial or endocardial connective tissue mantles contain more or less conspicuous elastic sheets. The elastic sheets which are situated on the outflow surface of the valve (auricular surface of the auriculoventricular valves; ventricular surface of semilunar valves) are generally the heavier and longer (Seipp). Both elastic layers thin out progressively as they approach the tip of the valve.

As a generalization it may be stated that while the separation of these valve layers is already seen in early fetal life, they become more and more clearly defined with advancing postnatal age periods (Veraguth). Furthermore, the differences in their extent, thickness, structure and distribution give to each cusp its individual characteristics.

## AORTIC VALVE

### *Topography*

The topographic relations to their attachments of each aortic cusp, indeed of various parts of each aortic cusp, show wide individual differences. This is due largely to the peculiar linking up of the left and posterior aortic cusps with the anterior leaflet of the mitral valve and the consequent deformation of the auricular myo-

one which is apposed to the left aortic cusp. The remaining pulmonary cusp is the anterior. The terminology of the auriculoventricular valve cusps needs no special explanation.

### METHODS AND MATERIAL

The descriptions which follow are based on an examination of 1000 normal human hearts. These were opened in the customary manner, the chambers washed out and packed, and the hearts fixed in a 10 per cent neutral formaldehyde sodium chloride solution.\* Occasionally a formaldehyde solution of Mueller's fluid † was used as a fixative and, if the specimens were very fresh (less than six hours postmortem), Bouin's fluid was employed. After fixation, suitable blocks were cut from the heart and at least two slides were stained from each block, one with hematoxylin and eosin, the other with Weigert's elastic and Van Gieson's connective tissue stains. Latterly, Masson's trichrome stains were used with excellent results.

For the hearts available during the latter part of these studies the standardized method for sections described by Gross, Antopol and Sacks was used in order to obtain comparable statistical material.

### GENERAL DESCRIPTION OF HUMAN HEART VALVES

Human heart valves have certain general features in common and yet sufficient individual differences to distinguish them sharply from one another. All the valves carry as their main backbone a dense collagenous layer which we propose to call the *Fibrosa* (Figs. 1 and 6). On its auricular aspect in the auriculoventricular valves (Fig. 8), as well as on its ventricular aspect in the semilunar valves (Fig. 6), the *Fibrosa* shows a looser structure which we propose to call the *Spongiosa*. In the semilunar cusps this looser structure may be so conspicuous as to constitute a sharply defined layer.

Each valve cusp is attached at its base to a more or less dense connective tissue structure called *Annulus Fibrosis*. This *Annulus*

\* Solution of formaldehyde, U. S. P., 10 parts; 1 per cent sodium chloride solution, 90 parts. This solution is rendered neutral with a weak alkali.

† The formaldehyde solution of Mueller (Formol-Mueller) is prepared as follows: potassium bichromate, 2 parts by weight; water, 100 parts; solution of formaldehyde, U. S. P., 10 parts. The "solution of formaldehyde" is added just before use.

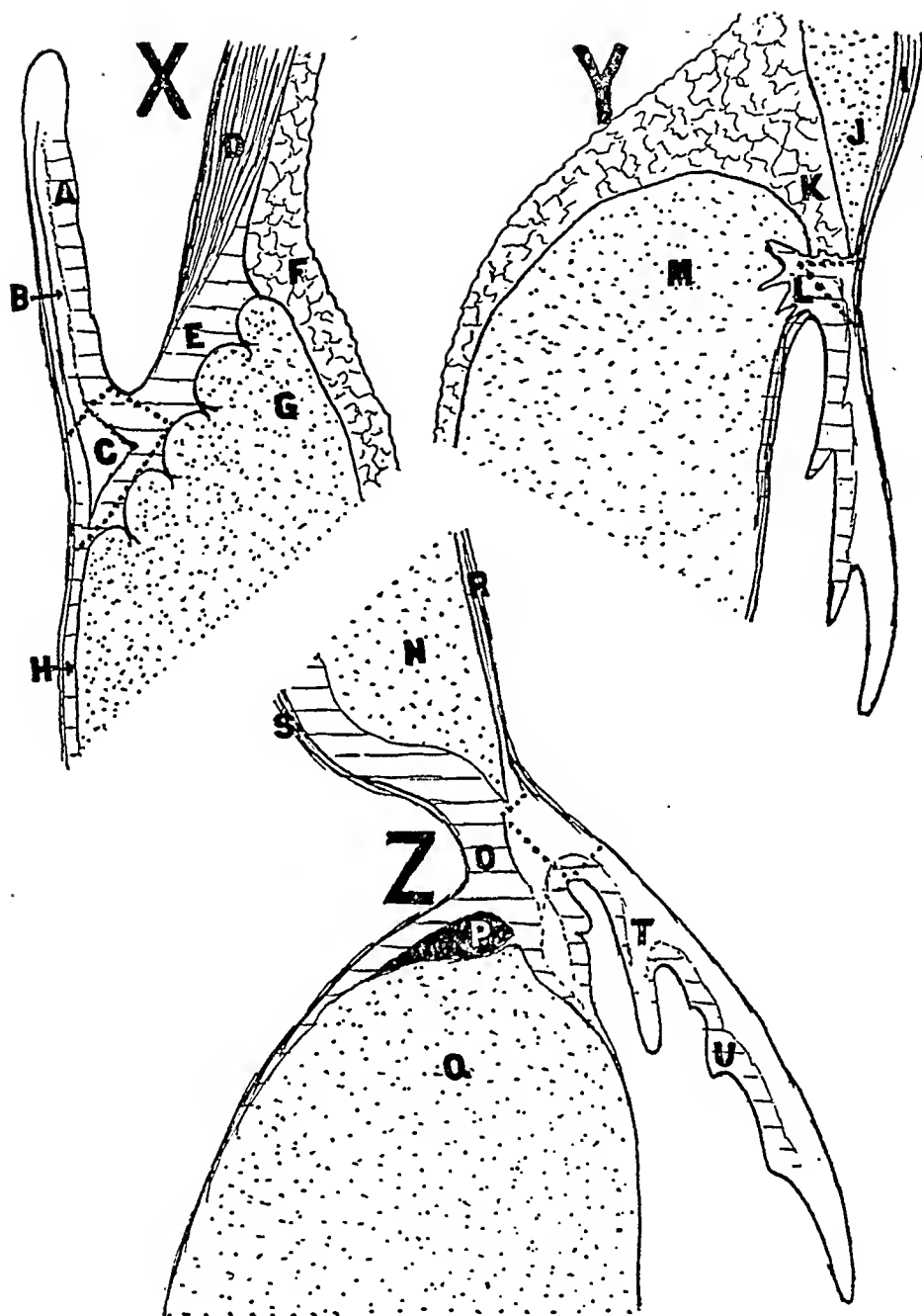
cardial and pericardial wedges which are, as it were, pressed in between them (Fig. 6). Other contributing irregularities in structure are the juxtaposition of the pulmonary myocardial conus and the presence of the *Septum Fibrosum* situated below the commissure between the right and posterior aortic cusps on the left side and the septal and anterior tricuspid leaflets on the right side.

As is well known, the aortic media is rich in elastic fibers. These exist as more or less parallel concentric sheets with, here and there, communicating transverse bars which include between them smooth muscle cells and connective tissue. As the media approaches the base of the aortic cusps it loses all but the most delicate of its elastic fibers and thus becomes the aortic *Annulus* (Fig. 1). Lewis and Grant have shown that for the greater part of the cusp, and in general, the aortic media ends in a wedge-shaped process whose apex is superficial to the *Annulus*. In the commissural region, however, the reverse of this takes place. They also found that in congenital bicuspid aortic valves the region of the fusion or absence of a commissure presents alterations in these relations. We have been able to confirm this and have noted in addition that as one approaches the commissures the wedge-shaped aortic termination changes into a more or less square pattern (Fig. 2) which again transforms itself into a wedge, now situated on the adventitial side of the aorta (Fig. 3). Furthermore, the commissural region is often marked by a slight thickening of the aorta at the level of the free edge of the cusps. Other parallel changes to be found in this region will be discussed in connection with the description of the *Sinus Pocket*\* to be given. Below the commissural junction the *Annulus* acquires elastic fibers and smooth muscle on its superficial (intimal) surface and thus again assumes the structure of aorta.

In the case of the *Right Aortic Cusp* (except that portion situated above the *Septum Fibrosum*) the aortic media generally changes into *Annulus* a short distance above the latter's insertion into the interventricular septum (Fig. 1). This insertion takes place by means of finger-like connective tissue processes which interdigitate with myocardial bundles. The main portion of the *Annulus*, however, makes a more or less obtuse-angled bend transversely and downward,† and

\* The more or less transverse portion of *Annulus* and intimal covering which lines the bottom of the sinus we designate *Sinus Pocket* (Fig. 5).

† In these descriptions, "transversely" will be considered in reference to the main anatomical axis of the heart; "downward" is synonymous with "toward the apex."



TEXT-FIG. 2. Diagrams of typical semilunar and auriculoventricular valve rings.

X. *Typical Semilunar Cusp*. A, fibrosa; B, valve spongiosa; C, ring spongiosa; D, aorta; E, annulus; F, pericardium; G, ventricular myocardium; H, subvalvular annulus.

Area enclosed by dotted lines is the valve ring.

Y. *Typical Auriculoventricular Cusp*. I, auricular endocardium; J, auricular myocardial wedge; K, pericardial wedge; L, annulus; M, ventricular myocardium.

Area enclosed by dotted lines is the valve ring.

Z. *Typical Septal Flap of Tricuspid Valve Section*. N, right auricular myocardial wedge; O, septum fibrosum; P, bundle of His; Q, interventricular septum; R, right auricular endocardium; S, left auricular endocardium; T, valve spongiosa dipping into chorda tendinea insertion; U, valve fibrosa.

Area enclosed by dotted lines is the valve ring.



(a) In the case of the left aortic cusp (Fig. 4) the *Annulus* is inserted into left ventricular wall instead of interventricular septum.

(b) No pulmonary conus exists adjacent to the pericardial mantle.

(c) Smooth muscle is less frequently seen in the *Subaicular Annulus* on section.

(d) If a block is cut through the middle of the valve, the left coronary artery or one of its branches will be seen embedded in pericardium not far from the arterio-annulus junction. If a section is cut more anteriorly, *i. e.*, close to the left-right commissure, the base of the pulmonary artery will be included; if cut more posteriorly, *i. e.*, close to the left-posterior commissure, the topographic relations will be similar to those to be described for the posterior cusp.

The topographic relations of the remaining portion of the left cusp, of the *Posterior Cusp* (Figs. 5 and 6), and of that part of the right cusp situated above the *Septum Fibrosum* are different in one important respect. Here, the aortic *Annulus* makes no bend as described above, but instead continues down to form the *Septum Fibrosum* or the *Fibrosa* layer of the anterior mitral leaflet, as the case may be. Because of this difference the corresponding aortic cusp *Rings* are brought much closer to the pericardial wedge, often being seated directly thereon instead of on myocardium. The pericardial wedge extends for a variable distance between *Annulus* and auricular myocardium in the case of the last-mentioned aortic cusps, at times passing below the level of the corresponding aortic *Ring*.\* From this description it will at once be grasped that there is a strategic proximity between these aortic *Rings* and the pericardium, so that an infection originating in the former could easily pass by contiguity into the pericardium, and *vice versa*.

In addition to the differences noted above we may mention that in the case of the posterior aortic cusp, the arterio-annulus junction is usually low, at times forming part of the *Sinus Pocket*.

Summarizing briefly these topographic relations of the aortic cusps, it is seen that they divide themselves into four types by which it is possible to recognize histologically the cusps from which the sections are cut as follows.

(a) The main portion of the right cusp has the "U" type of insertion in which the aorta forms one arm of the "U," the cusp the other arm, and the base of the "U" sits on the interventricular septum and forms the bottom of the *Sinus Pocket*. In this "U" type of insertion the valve *Ring* sits on myocardium

\* Occasionally the Arteria Anastomotica Magna (Kugel) may be seen in the pericardial wedge.

riding atop the interventricular septum makes another bend abruptly cephalad as it reaches the ventricular cavity. The region of the cephalad bend is the *Ring*. Here fibers of the *Annulus* split off into two bundles to surround the *Ring Spongiosa*. The bundle which rounds the *Sinus Pocket* and enters the valve flap to make up its main support, the *Fibrosa*, is generally the larger one. The smaller bundle courses under the *Ring Spongiosa* and continues for a short distance down the inner surface of the ventricle under the endocardium, as a thin fibrous tissue sheet which we call the *Subvalvular Annulus*. The endocardial layer clothing the *Subvalvular Annulus* generally shows smooth muscle cells on section. It is to be observed that for the greater part of its insertion the *Ring* of the right aortic cusp sits on myocardium and has no direct connection with the pericardial wedge. However, here as in all other semilunar cusps, the *Ring* insertion lies almost directly over the pericardial wedge in the region of the commissure.

The line at which the *Annulus* meets the interventricular septum is generally clothed by pericardial tissue, usually fat, which serves to separate this arterio-annulus junction from the adjacent myocardium of the pulmonary conus, the latter being a direct muscular continuation of the interventricular septum. Particular attention should be paid to the presence of the pulmonary conus in this section, since it forms an absolute means of identifying this cusp. Furthermore, if the section is cut high enough, it is possible to distinguish the anterior half of the right aortic cusp from the posterior half. The former section contains the root of the pulmonary artery, the latter will show a cross-section of the right coronary artery.

It has already been mentioned that as one passes from the middle of the valve toward a commissure, alterations occur in the aortic media-annulus relations. In addition, it is to be noted that the *Sinus Pocket* formed by the *Annulus* becomes progressively smaller and the myocardium-annulus insertion line on cross-section assumes a horizontal direction instead of sloping from above downward (considered from the pericardial to the inner surface) as is the case in the middle of the cusp (Figs. 1, 2 and 3). These rules apply to all the aortic and pulmonary cusps.

With the following modifications, the description given for the topographic relations of the *Right Aortic Cusp* holds for the *Left Aortic Cusp* except the portion situated above the anterior mitral leaflet.

The ridge constituting the line of closure of the aortic cusps consists of an abrupt thickening of the *Ventricularis* layer. This thickening is made up of closely packed vertically directed fibers of connective tissue interspersed with elastic fibrillae. The whole structure rests on a somewhat condensed band of *Elastica* which separates it from the remainder of the valve substance. In the center of the valve this closure line assumes nodular proportions and is called Nodulus Arantii (Fig. 7). In advancing age periods these Noduli become increasingly prominent, hardened and elastified threads often breaking off them at one end to float as streamers in the blood current. These form the so-called Lamblian excrescences. They are of interest in that they have been confused with healed verrucae.

The *Ventricularis* as a whole is rather poor in cells, though fibroblasts and occasional large mononuclear cells with scanty protoplasm can be seen scattered throughout it. These cells are more plentiful in the earlier age periods. This decreasing cellularity with increasing age is found in all the valve layers of the semilunar, as well as auriculoventricular, cusps.

*Spongiosa*: It has already been mentioned that the backbone of each valve leaflet consists of a dense collagenous layer and that in the aortic cusps the ventricular aspect of this layer gives way to a much looser structure which we designated *Spongiosa*. In a fetus of crown-rump length 12 cm. the *Spongiosa* already begins to be discernible. In the average adult of the second and third decade it consists of a very loose connective tissue layer which extends from the *Ring* (*Ring Spongiosa*) for a variable distance almost to the tip of the valve (Fig. 6). At the *Ring* the *Spongiosa* has a triangular shape limited below by *Annulus*, above by *Fibrosa* and toward the ventricular surface by *Ventricularis*. Interspersed in the loose connective tissue of the *Spongiosa* with its scarce fibroblasts and other mononuclear cells there can be found irregular clumps of dense collagen fibers, as well as a delicate feltwork of elastic fibrillae which are continuous on the one hand with those springing from the *Ventricularis* and on the other hand with rare elastic fibrillae running between the collagenous bundles of the *Fibrosa* (Fig. 9).

*Fibrosa*: Under the heading of "Topography" it has already been mentioned that the root of the aorta loses its musculature and most of its elastic fibers, thus transforming itself into *Annulus*, and that much of this collagenous *Annulus* curves around the *Sinus Pocket*

through the intermediary of a band of *Annulus*. The arterio-annulus junction is clothed by pericardium and the pulmonary myocardial conus lies adjacent to it.

(b) The main portion of the left cusp also has the "U" type of insertion. Here, the distinctive features are the absence of adjacent pulmonary conus and the frequent presence of coronary artery embedded in free visceral pericardium.\*

(c) Continuing the scheme of using letters to represent physical configuration it may be said that the main portion of the posterior aortic cusp has the "Y" type of insertion. Here, the aortic *Annulus* continues as anterior mitral valve *Fibrosa* and forms the stem of the "Y." The posterior aortic *Ring* usually sits on pericardial wedge.

(d) The portions of right and posterior cusps participating in the right-posterior commissure also have the "Y" type of insertion but here the *Annulus*, in the form of the *Septum Fibrosum* (stem of the "Y"), eventually inserts into interventricular septum. The insertion is by means of connective tissue bundles interdigitating with myocardium.

### Histology

For practical purposes the description which follows corresponds to an average aortic cusp of between the third and fourth decades. The age period changes will be briefly commented on below.

*Ventricularis*: The aortic cusp is clothed on its ventricular aspect by a fibro-elastic layer of varying degrees of density (Figs. 1 and 9). This layer, which we propose to call *Ventricularis*, is virtually a continuation of the ventricular endocardium, except for the posterior and left aortic cusps, where it is a continuation of the *Ventricularis* of the anterior flap of the mitral valve. This will be taken up subsequently. Histologically, the *Ventricularis* is seen to be covered by a single row of flat endothelial cells with round or oval nuclei and to consist of vertically (*i.e.*, parallel with the long axis of the body) directed connective tissue fibers interspersed with delicate elastic lamellae. The latter tend to become thicker and bunched together toward the ventricular surface, thus forming the *Ventricularis Elastica*.

It has been claimed by various authors that the elastic fibers in the valves do not appear until after birth. Nevertheless, we have found them in the *Ventricularis* at the proximal portion of the valve flap in 19 cm. (crown-rump) fetuses.

\* The term "free visceral pericardium" denotes the absence of adjacent cardiac structure. Note that the left aortic cusp is the only one which shows free visceral pericardium on section at the *Ring* level.

aorta, the metamorphosis of pulmonary artery into *Annulus* takes place at, or slightly above, the level of the subjacent ventricular myocardium. The relations of pulmonary artery to *Annulus* are similar to those described for the aorta and the rules laid down concerning the slope of the *Annulus* and the changes which take place on approaching the commissure hold for the pulmonary cusps.

TABLE I

*Comparison of Histological and Topographical Features of Pulmonary and Aortic Cusps*

<i>Pulmonary Cusps</i>	<i>Aortic Cusps</i>
* Pulmonary artery delicate.	* Aorta thick.
* Various layers delicate.	* Layers on the whole thick.
<i>Fibrosa</i> frequently shows transverse ridge-like projections into <i>Sinus Pockel</i> .	Ridge-like projections less frequent.
<i>Ring Spongiosa</i> often small and fibrillar. Not infrequently absent from section.	<i>Ring Spongiosa</i> generally larger. Seldom fibrillar, more often spongy. Seldom absent from section.
<i>Valve Spongiosa</i> less frequently definite. Character similar to <i>Ring Spongiosa</i> .	<i>Valve Spongiosa</i> sharply defined when present. Character similar to <i>Ring Spongiosa</i> .
Smooth muscle in <i>Ventricularis</i> rare in any cusp section.	Smooth muscle in <i>Ventricularis</i> not infrequent, especially in <i>Ventricularis</i> of right aortic cusp.
* <i>Subvalvular Annulus</i> is absent or very delicate.	* <i>Subvalvular Annulus</i> conspicuous.
* Interdigitations of <i>Annulus</i> with myocardium generally delicate.	* Interdigitations coarser, increasing with age periods.

(The items marked by asterisks demonstrate the more characteristic differences.)

The main differences between the pulmonary and aortic valve sections are brought out in Table I. The items marked by asterisks demonstrate the more characteristic differences. In addition to the points brought out in the table, it is to be noted that in contrast to the aortic valve where the left cusp section is the only one to show "free visceral pericardium," the latter is present on the anterior and right pulmonary cusps.

(Valsalva) to ascend the valve leaflet as its *Fibrosa*. In rounding the *Sinus Pocket* the collagenous bundles change their general direction and structure. In the *Annulus* they are in the form of interlacing whorls. Under the *Sinus Pocket* the superficial bundles take on a course more or less parallel to the bottom of the "U" and, as the *Fibrosa* proper, they run mainly in a transverse direction.

In the crypts between the collagenous bundles there are to be found very delicate elastic fibrillae, as well as mononuclear cells with round dense nuclei and rather scant protoplasm. The *Fibrosa* ascends toward the tip of the cusp. In most instances, however, the arterial aspect of the tip of the *Fibrosa* consists of a much looser, at times myxomatous, structure, not unlike the *Spongiosa* in character (Fig. 1).

The *Fibrosa* is more or less sharply limited on its ventricular aspect by the looser *Spongiosa* and on its arterial aspect by the delicate *Arterialis* about to be described.

*Arterialis*: As one follows the more superficial elastic fibers down the intima of the aorta toward the cusps, they are seen to fuse into one or more bands which round the *Sinus Pocket* superficially and ascend the arterial surface of the aortic cusps as a delicate elastic layer. This rapidly thins itself out, often disappearing about one third of the way up the valve. Before progressive (hyperplastic) changes have taken place, this elastic lamella is clothed by a layer of flat endothelial cells and is separated from the adjacent *Fibrosa* by a very sparse, loose connective tissue containing extremely delicate elastic fibrillae, as well as mononuclear cells poor in cytoplasm with darkly staining nuclei. Normally, and in younger individuals, the endothelial cells sit practically directly on this elastic layer.

As has been mentioned before, the *Arterialis Elastica* is shorter and far more delicate than the corresponding *Ventricularis Elastica* (Figs. 5 and 9).

## PULMONARY VALVE

### *Topography*

The topography of the pulmonary cusps is very simple. The general plan is the "U" type of insertion, as described for the aortic cusps. Because of this there is no direct insertion of pulmonary *Ring* on pericardium, except at the commissural junctions. As in the

In later age periods the prominent *Noduli Arantii* of the aortic valve form a characteristic difference from the corresponding, more delicate, so-called *Noduli Morgagni* of the pulmonary cusps.

#### GENERAL CONSIDERATIONS CONCERNING THE AURICULO- VENTRICULAR VALVES

It is a relatively simple matter to set definite limits to the extent of the semilunar valve cusps. In a previous paragraph it was suggested that the valve *Rings* should be considered to constitute their proximal end. This gives one a fairly concrete point of departure, inasmuch as the valve *Ring* itself is almost invariably limited proximally by subjacent *Annulus* (Fig. 1). In any event, shifting the imaginary line of semilunar cusp insertion slightly one way or another is, in so far as one can see, of no great moment.

This, however, is distinctly different in the case of the auriculo-ventricular cusps for, as indicated earlier in this paper, one's conception of the limits of the cusps inevitably carries with it the question of the existence of myocardium, and because of this, of blood vessels in the valve. A glance at a typical cusp (Fig. 6) will make this point clear. If arrow "A" were to be considered the proximal end of the cusp, the auricular myocardial wedge would be included and it could therefore be said on this basis that many of the auriculo-ventricular cusps have myocardium. Further, since myocardium is always supplied by blood vessels, it follows according to this definition that these auriculoventricular cusps possess blood vessels. If, on the other hand, arrow "B" is to be considered the proximal end of the cusps (and this is the definition which we favor), the myocardium and its blood supply ceases to be a factor in the argument and the question of the existence of blood vessels in human heart valves involves consideration of only the fibro-elastic portion — an entirely different matter as will be seen. It seems to us that this is the crux of the discussion on whether myocardium exists in the auriculoventricular valves and it is on this very point that a large and confusing literature has sprung up. Various authors have chosen arbitrary limits for the origin of the auriculoventricular valves, particularly of the anterior mitral cusp, and have reported abundant or sparse auricular musculature extending for a greater or lesser distance down the valve according to their conception as to whether

The individual characteristics of the several pulmonary cusps are as follows.

The *Left Cusp* (Fig. 11) is inserted on to the anterior portion of the interventricular septum; as a consequence of this a section through the middle of this cusp shows the double myocardial crest characteristic of the latter. If cut more toward the left, it will show a large transverse section of ventricular wall, possible with visceral pericardium. If cut toward the right, adjacent aortic root will be seen. These sections show no subvalvular *Annulus* but are apt to have left coronary artery present.

The *Anterior Cusp* (Fig. 12) is seated on the delicate anterior wall of the pulmonary conus. In this region the pericardial mantle is generally very thin. If subvalvular *Annulus* is present in the pulmonary valve it will generally be found in the anterior cusp section as a delicate layer extending several millimeters below the valve *Ring*. This, together with the very slender visceral pericardial layer mentioned above, is characteristic of the cusp.

The *Right Cusp* (Fig. 13) is seated on the more supple right wall of the pulmonary conus. Here the pericardial fat is generally quite thick. These features and the absence usually of *Subvalvular Annulus* are characteristic of this cusp. If a section is cut more posteriorly, adjacent aorta may be seen.

### *Histology*

The histological differences between pulmonary and aortic cusps are much less obvious than are the topographic. In general, as mentioned before, the layers are more delicate in the former. The *Ventricularis* and *Arterialis* layers are generally so thin as to make the *Fibrosa* more conspicuous by contrast.

Whereas in the aortic cusps the *Ring* and *Valve Spongiosa* are, as it were, sharply scooped out of the *Fibrosa* and filled with a loose jelly-like tissue made up of transversely coursing collagen and elastic fibrillae with stellate cells, in the pulmonary cusps the separation of this layer from the *Fibrosa* is usually not so sharp, fragments of collagen being scattered throughout (*cf.* Figs. 6 and 12). Elastic fibrillae are not seen so often until later age periods and the connective tissue feltwork is often denser.



*Rings* (Text-Fig. 2). In the case of all the auriculoventricular cusps, excepting the septal flap of the tricuspid, the *Ring* may be arbitrarily considered as an inverted pyramid-shaped portion of *Annulus* in the immediate vicinity of the auricular myocardial wedge apex, limited above by the line referred to, which constitutes the base of this inverted pyramid, and internally by the endocardium. We have found it convenient to consider the pyramid more or less equal-sided and have taken the width of the valve as a rough measure of each side. In contrast to all the other auriculoventricular valve cusps, the septal flap of the tricuspid, as will be noted in Fig. 16, is generally inserted onto *Annulus Fibrosis* or interventricular septum through the intermediary of a wide base. Here the *Ring* may be conveniently considered as roughly rectangular in shape. The upper limit is the transverse line drawn through the apex of the auricular wedge; the lower limit is a similar line drawn at right angles to the endocardium at the level of the *Sinus Pocket*; the outer limit is in an imaginary line drawn parallel to the endocardium at a distance from it approximately equal to the width of the valve; the inner limit is the endocardium.

Contrary to what is found in the semilunar valves, the auriculoventricular cusps seldom have a clearly delimited valve or *Ring Spongiosa*. In the region of the apex of the auricular myocardial wedge the *Fibrosa* generally shows a looser structure of its collagenous bundles. For the sake of uniform terminology this layer should also be referred to as *Spongiosa*. It may at times be seen extending along the auricular aspect of the *Fibrosa* toward the valve tip (Figs. 8 and 16). The layer is generally more conspicuous opposite the insertions of the chordae tendineae where it can be seen at times dipping into the latter in the form of a wedge. Both here and at the *Ring* the *Spongiosa* may acquire considerable fat during the course of the degenerative involutionary changes attendant on later age periods.

The most conspicuous feature of the auriculoventricular valve structure is the extremely dense, broad *Fibrosa* which constitutes the bulk of the cusp. As in the semilunar cusps, the *Fibrosa* not infrequently transforms itself into a looser, sometimes myxomatous, structure as it approaches the tip. The chordae tendineae may be considered as dense collagenous prolongations of the *Fibrosa* layer which link the latter to the papillary muscles or ventricular myocardium.

the base of the valve was higher or lower in the auricle (Kürschner, Reid, Joseph, Gussenbauer, Beitzke, Hoessli, Tandler, De Castro). The significance of the relatively infrequent occurrence of ventricular musculature in the base of the tricuspid valve will be discussed later.

It is obvious from what has been said that the presence or absence of myocardium in the auriculoventricular valves must be settled before one can intelligently discuss the existence of blood vessels in valves. Furthermore, inasmuch as the latter question is intimately bound up with the embolic theory of endocarditis, a clear definition of terms becomes not only desirable but absolutely essential. When one considers the question of the pathogenesis of valvulitis one is primarily concerned with the membranous structure. This very practical reason alone should justify a definite limitation of the valve to the fibro-elastic portion. But there are other reasons as well. We have seen that the valve *Ring* is a natural and simple limit to be set for the extent of the semilunar cusps. It is desirable that, if possible, a uniform conception be held for all valve cusps and that therefore the extent of the auriculoventricular valves should be limited in the same manner. Furthermore, it does not seem to be more unreasonable to exclude auricular or ventricular myocardium from our definition of valve leaflet than it does, for example, to exclude sclera from our conception of what constitutes cornea. Apparently, Weber and DeGuy appreciated this source of confusion in the case of the anterior mitral cusp and attempted to circumvent the difficulties by naming the entire structure "*la région mitro-aortique*" and dividing it into three zones, *viz.*: (1) *la zone supérieure ou sigmoïdienne . . . limitée . . . en bas par trois lignes . . . d'insertion des valvules aortiques*; (2) *la zone moyenne ou mitro-auriculaire, limitée . . . en bas par une ligne transversale correspondant à la partie terminale des fibres musculaires venant de l'oreillette . . .*; and (3) *la zone inférieure ou grande valve mitrale. . .*

In view of all the considerations mentioned above and for many equally important reasons which need not be entered into here, we propose to use the valve *Ring* to set the proximal limit of the auriculoventricular cusps. Since all the auriculoventricular cusps have inserted into them a wedge of auricular myocardium, a line drawn through the apex of this wedge at right angles to the auricular endocardium may be considered an excellent and simple means of defining in turn the proximal limit of the auriculoventricular valve

aortic *Annulus* continues down the ventricular face of the auricular myocardial wedge and after reaching the tip of the latter becomes successively anterior mitral cusp *Ring* and valve *Fibrosa*. The portion of *Annulus* which extends between the aortic and mitral *Rings* we call *Mitral-Aortic Intervalvular Fibrosa*. It is well to note that this structure belongs neither to aortic nor to mitral cusps. For embryogenetic and anatomical reasons this, like the myocardial wedge, should be considered part of the auricle.

Further points of importance are the characteristic auricular myocardial wedge clothed by thick left auricular endocardium and the typical structure of aortic cusp (left or posterior) superimposed.

### *Histology*

*Auricularis*: As is well known, the left auricular endocardium is made up of dense fibrous and elastic sheets interspersed with smooth muscle bundles which are generally situated close to the subendocardium. As the elastic fibers are traced toward the mitral valve it is seen that the outermost lamellae (*i. e.*, those toward the subendocardium) tend to fuse into a more or less condensed stratum. In the region of the apex of the auricular myocardial wedge many of the remaining elastic lamellae fuse into this lower condensed stratum. In the later age periods, the innermost elastic sheets also show condensations which fuse with the subendocardial strata near the auricular myocardial wedge apex. The net result is that the number of elastic lamellae is considerably reduced and concentrated. This concentration zone continues down the auricular surface of the mitral valve for a variable distance toward the tip. Interspersed among the elastic fibers there can be seen stretches of collagen.

In the case of the anterior mitral cusp, a continuation of the endocardial smooth muscle can usually be traced down the *Auricularis* layer, clothed by *Auricularis* elastic lamellae. The smooth muscle can become so prominent as to form, in some cases, approximately one-fifth of the entire thickness of the valve leaflet. For about two-thirds of the way down the cusp the elastic lamellae can be seen to lie almost immediately beneath the endothelial-covered surface. However, as the closure line of the valve is approached at times the bulk of the elastic lamellae begins to occupy a slightly less superficial position, being covered by a thicker and wider band of

In leaving the *Fibrosa*, the chordae tendineae carry with them a light mantle of the *Ventricularis* covering the valve leaflet.

With the above mentioned points in mind, the typical auriculo-ventricular cusp section shows the following topographic features (Fig. 9).

An auricular portion consisting of endocardium and subjacent myocardium ends in a wedge.

Linking this wedge to ventricular myocardium and interdigitating with the latter there is a dense collagenous *Annulus*.

On the external surface, pericardium clothes this auriculoventricular junction and sends a wedge of adipose tissue to insert into the *Annulus*. The proximity of this pericardial wedge to the valve *Ring* and the frequency with which the latter is the seat of inflammation are matters of considerable interest from the point of view of pathogenesis.

The *Annulus* merges imperceptibly with the valve *Ring* at the level of the myocardial wedge.\* The *Ring* in turn gives way to valve *Fibrosa* and this continues for a variable distance toward the valve tip.

The auricular endocardium continues beyond the myocardial wedge tip down the valve as its *Auricularis* layer. Similarly, too, the ventricular endocardium passes under the *Valve Pocket* clothing the ventricular surface of the *Annulus*, rounds the latter and spreads itself over the outer surface of the cusp as the *Ventricularis*.

## MITRAL VALVE

### *Topography*

The topography of the typical auriculoventricular valve has already been described and the description given is directly applicable to the *Posterior Mitral Cusp* (Fig. 8). A section through this region shows the characteristic dome-shaped crest of the left ventricular wall, the thick left auricular endocardial layer, the not infrequent passage of the left auricular myocardial wedge below the level of the ventricular crest and the presence, generally, of left coronary circumflex artery in the *Free Pericardium* of the auriculoventricular groove. At times a short subvalvular extension of the *Annulus* may be seen on the ventricular wall below the valve pocket.

The *Anterior (Aortic) Mitral Cusp* is unique in structure (Fig. 6). In describing the posterior aortic cusp it was mentioned that it falls into the category of the "Y" type of *Annulus* insertion, *i. e.*, the

\* At times the auricular myocardial wedge may send several finger-like strands of tissue for as much as 1 cm. down the valve cusp. This places the *Ring* unusually low, but it is better so considered than to change the definition and thus confuse more important issues.

toplasm lying between its bundles is generally almost entirely free of elastic fibrillae (Fig. 10). It extends toward the cusp tip where it becomes continuous with the spongy structure referred to above.

The *Fibrosa* of the *anterior mitral cusp* is a direct continuation of the *Mitral-Aortic Intervallvular Fibrosa* and the general direction of its fibers is transverse.

*Ventricularis*: In the case of the *Posterior Mitral Cusp*, the endocardial fibro-elastic covering of the ventricle curves round the valve pocket and descends for a variable distance toward the cusp tip forming its *Ventricularis*. The elastic fibers are generally considerably more delicate and shorter than the corresponding ones on the *Auricularis* layer. They are covered by the same type of endothelium as described for the *Auricularis* layer and between the elastic lamellae and the *Fibrosa* there is to be found a small amount of relatively acellular loose gelatinous tissue. Under certain conditions the *Ventricularis* layer may become somewhat thickened.

In the case of the *Anterior Mitral Cusp* (Fig. 6), this layer is a direct continuation of the subaortic endocardial tissue, the latter in turn being a prolongation of the semilunar cusp *Ventricularis*. Furthermore, in this cusp the *Ventricularis* shows many dense bundles of elastic tissue, at times much more conspicuous than the *Auricularis* bundles. This is particularly well seen in the younger age periods where the *Ventricularis Elastica* carries with it a very definite structure of longitudinal running collagenous bundles. As in the case of the mitral posterior, this *Ventricularis Elastica* is generally shorter than the *Auricularis Elastica*.

## TRICUSPID VALVE

### *Topography*

The topography of the tricuspid leaflets follows closely the general description given for the auriculoventricular valves. However, they can be very easily distinguished from the mitral flaps because of their greater delicacy in structure and other peculiarities of their own about to be described. As in the case of the mitral cusps the general plan is that of an auricular endocardial and myocardial wedge which is joined to a ventricular crest through the intermediary of *Annulus*, the tricuspid cusps springing from this *Annulus*.

The *Anterior Flap*, the longest of the three, shows an extremely

dense connective tissue which may in turn carry more delicate strands of elastic tissue.

The most superficial covering of the auricular surface of the valve is the endothelial layer referred to above. This consists of flat cells with scanty protoplasm and moderately dense nuclei. Between this endothelial layer and the most superficial elastic band there is an almost imperceptible jelly-like zone containing an occasional mononuclear cell.

The tips of the mitral leaflets are generally of a more gelatinous, embryonal consistency (Figs. 6 and 8). The *Auricularis* layer described, together with the others about to be described, merge imperceptibly with this loose embryonal tissue of the valve tip. The latter consists of a network of stellate cells with scanty protoplasm lying in a matrix poor in collagenous fibrillae and staining at times faintly bluish with hematoxylin. As in the semilunar cusps the *Auricularis* layer is relatively poor in cells.

*Spongiosa*: The left auricular subendocardium consists of dense bundles of collagen interspersed with thick elastic fibers. A continuation of this structure mingles with the outer looser layer of the *Fibrosa* to form the essential substance of the mitral valve *Spongiosa*. In the case of the posterior cusp the structure is generally much looser than that of the anterior and, as mentioned before, opposite the insertions of chordae tendineae it may assume an appearance which is almost indistinguishable from that seen in the semilunar cusp *Spongiosa*. That is, small collagenous bundles may be seen irregularly interspersed with a feltwork of delicate elastic fibrillae, a very loose ground substance and scatterings of mononuclear cells. In the region of the *Ring* the elastic fibrillae are generally coarse. At or near the cusp tip the *Spongiosa* layer merges imperceptibly with the other layers.

*Fibrosa*: In the case of the *Posterior Mitral Cusp*, it has already been mentioned that the *Annulus* interdigitates with myocardium and generally continues as an extension under the ventricular subendocardium. These collagenous fibers, on the whole, run parallel to the ventricular surface, round the valve pocket and continue into the valve substance to form the *Fibrosa* layer (Fig. 8). Here the fibers run parallel to the chordae tendineae. Indeed, they form an unbroken continuation with the main body of the latter. The collagenous *Fibrosa* which is dense and shows mononuclear cells with scant pro-

myocardium ends in a wedge whose apex is superficial to the *Septum Fibrosum*. The general slope of the latter is usually from above downward, considered from left to right.

In the region of the *Septum Fibrosum* the ventricular myocardium ends in a fairly regular dome-shaped crest. As one proceeds backward, however, the dome shape can at times be seen to be replaced by a sloping surface whose highest point is toward the left ventricle. It will be seen from this that the general slope of the *Septum Fibrosum* is also continued in the myocardial portion of the interauricular septum.

The pericardial wedge as such is present in an abortive form only in the posterior sections of this cusp where fragments of fatty tissue can be seen to surround the auriculoventricular node. The continuation of the latter in the form of the bundle of His can be readily seen in this section. As in the other two tricuspid sections, *Subvalvular Annulus* is inconspicuous. In the case of all three sections the ventricular myocardial interdigitations are slender.

### *Histology*

The histology of the tricuspid leaflets is similar to that of the mitral valve posterior. The following features, however, should be noted.

The endocardium of the right auricle shows a considerable abbreviation of structure over that of the left auricle. It is generally thinnest over the anterior cusp. The latter tends to be rather dense in structure and shows the looser myxomatous tip less frequently than the other cusps. The general direction of the collagenous bundles is similar to that described for the mitral posterior cusp. There is often a greater difficulty in discerning the *Spongiosa* layer in the tricuspid cusps. The general distribution of the elastic lamellae and the cytology shows no fundamental differences from what has already been described. One peculiarity of the tricuspid leaflets to which attention has already been drawn in the literature is the fact that ventricular myocardial bundles are not infrequently found inserted fairly low into the base of the valve. This is particularly true for the posterior cusp and is found much less frequently in the septal cusp. In spite of the numerous reports published on this point it does not seem that the findings of these muscular bundles is of ap-

delicate auricular endocardium with scattered smooth muscle cells. The peculiarity of the ventricular crest easily distinguished this section because the myocardium tends to assume a trough shape, the concavity of which points toward the auricles (Fig. 14). A rather abundant free pericardial wedge fills in the space between this trough and the right auricular myocardial wedge. This wedge carries the right circumflex coronary artery. A few strands of subvalvular *Annulus* can occasionally be seen in the section.

The *Posterior Tricuspid* section differs from the anterior in several respects. Here, the auricular endocardium retains the slender structure characteristic of right auricle but is generally thicker than that seen in the anterior tricuspid section. The smooth muscle cells are generally more abundant. While the septal crest is seldom as rounded, dome-shaped and regular as in the mitral posterior section, the characteristic trough structure of the anterior tricuspid is rarely present — a more bulky, often irregular dome-shaped myocardial crest taking its place (Fig. 15). The separation between the auricular and ventricular myocardium is generally more marked in this section, the *Annulus* showing a neck-like extension. At times this neck-like extension may consist partly of myocardial bundles. There is seldom *Subvalvular Annulus* to be seen. The pericardial wedge is moderate in amount and generally carries a branch of coronary artery. The flap itself is shorter than the anterior.

The *Septal Flap* is the shortest of the three and its situation on the septum is so characteristic that one can easily distinguish this cusp from the others, as well as determine the particular point at which the section was cut (Fig. 16). The most anterior portion of this cusp is inserted on *Septum Fibrosum* so that the *Annulus* of this cusp becomes continuous with it. Since, further, the *Septum Fibrosum* is also continuous with the *Annulus* of the aortic cusps, there exists thus a collagenous linkage between mitral, aortic and tricuspid *Rings*. As one traces the septal cusp backward, however, its insertion line passes onto the top of the interventricular crest and occupies a progressively lower (caudad) site as it extends posteriorly. Thus, by estimating the approximate distance between the tricuspid *Ring* and the top of the septal crest, one can fairly well establish the position of the section.

The auricular endocardium is generally of about the same structure as that seen in the posterior tricuspid section. The auricular



cardium. Considered in this way, and we suggest that this is a logical and simple definition, the question of the existence of blood vessels in valves ceases to be one of major importance. Certainly if they occur at all they are most infrequent.

It may be said in passing that the practice of comparing human heart valves to those of lower animals is one which is very prone to lead one into erroneous conclusions.

### THE QUESTION OF THE EXISTENCE OF BLOOD VESSELS IN THE RINGS

In describing the various valve *Rings* we attempted to picture the cellular and tissue components which make up their structure. No mention was made of the presence or absence of blood vessels in these sites. This omission was deliberate and was done in order to simplify the description. Actually, in a statistical analysis made for this purpose on 100 hearts from various age periods, capillaries only were found in the *Rings* with the following frequency:

Anterior mitral valve <i>Ring</i> .....	1 per cent
Posterior " " " .....	2 " "
Aortic " " " .....	0 " "
Tricuspid " " " .....	14 " "
Pulmonary " " " .....	7 " "

Excluding the tricuspid *Ring* from consideration for a moment, it may be stated that in not a single heart were vessels found in more than one of the other *Rings*. This fact, together with others to be discussed, seems to us to be of considerable importance.

It will be observed that capillaries are not infrequently found in the tricuspid *Ring* (14 per cent), particularly in the septal flap *Ring*. These capillaries are generally in the form of rather large thin-walled channels. They do not resemble granulation tissue capillaries in any respect.

The capillaries found in the other *Rings* are generally few, usually near to the subjacent myocardium, small and circular on cross-section, not surrounded by inflammatory cells, and in structure easily differentiated from granulation tissue capillaries.

It will be obvious from the above description that the great infrequency of capillaries, particularly in the mitral and aortic *Rings*, their relative infrequency in the other *Rings*, their normal appear-

preciable significance. The bundles generally do not descend deep into the cusps and are nothing more than a slight exaggeration of the natural infolding of the ventricular myocardial tube with that portion of the auricular canal which is concerned with the formation of auriculoventricular cusps. The same explanation accounts for the not infrequently low position of the auricular myocardial wedge in these sections.

#### THE QUESTION OF THE EXISTENCE OF BLOOD VESSELS IN THE VALVES

It will be noted that in the description of the various valves given in this report blood vessels were not mentioned. We have deliberately avoided taking up the very controversial question as to the existence of blood vessels in normal valves largely because it can not be done justice, without going into very lengthy detail. Briefly considered, it may be said that there are three schools of thought on this subject: One group holds that human heart valves have no blood vessels; another believes that human heart valves are supplied with blood vessels in at least a considerable proportion of cases and that failure to demonstrate them by many observers is due to faulty technique; the third group believes that blood vessels occur at times in postnatal human heart valves and that when they occur they probably represent fetal remnants.

Without desiring to enter into controversy here, we may say that we are rather doubtful at present as to whether blood vessels occur, at any rate with any degree of frequency, in *normal* human heart valves, providing we have a very clear understanding as to what we consider *normal*.

In previous reports evidence was adduced by us which pointed to the fact that in at least 2 per cent of a group of 700 individuals who came to autopsy in the general hospitals from which our material was obtained, blood vessels were found in some valves of what appeared to be otherwise normal hearts. Our present scepticism is due to the fact that the results of our more recent investigations have caused us to doubt the normality of many of the hearts which we previously considered normal. Furthermore, as indicated in the description given above, our definition of the *Ring*, which is the proximal termination of the valve cusp, automatically excluded myo-

investigators believe them to be vestigial remnants of valve vasculature. Others consider them endocardial sack-like infoldings. Another possibility which we would like to suggest is that during the course of development endothelial cells may be invaginated into the substance of the cusp, become nipped off and, because of their angioblastic properties, develop aborted vessels or vascular cyst-like spaces. The contained blood cells themselves may be of similar origin.

### AGE-PERIOD CHANGES IN VALVES

The human heart valves, like other structures in the body, continue to show evolutionary and involutionary changes after birth. What the significance of these changes may be in respect to the production of disease processes one can only surmise. It is well to bear in mind, however, that with advancing age periods the various layers of the valves previously described become progressively poorer in cells and take on the following changes: The strata become increasingly well defined; the semilunar cusp *Spongiosa* becomes more and more fibrous and elastified; the auriculoventricular valve *Ring Spongiosa* and the *Spongiosa* situated opposite the chordae tendineae insertions become loose and often the seat of fat deposits; the elastic membranes become heavier and longer; the *Auricularis* and often the *Ventricularis* layers become appreciably denser, more collagenous and thickened; and the collagenous *Fibrosa* undergoes degenerative lipid changes. The point last mentioned is inevitably associated with the arteriosclerotic calcium salt deposition of the later age periods. Particularly is this true of the lime changes which occur in the *Annulus* of the valve *Rings* (Mönckeberg).

### SUMMARY

There has been described in this paper in some detail the normal histology and topography of the individual cusps of the valves in the human heart. It has been shown that these have a number of characteristics in common and yet sufficient individual differences to distinguish them from one another. The desirability of sharply delimiting the different cusps has been discussed and a method of accomplishing this has been suggested. This method is calculated to clear up the confusion which exists on the question of the presence of

ance, the absence of inflammatory cells, the fact that the occurrence of capillaries in more than one *Ring* at the same time (excluding the tricuspid) must be very rare, together with the absence of arterioles or large vessels in these sites, make any definite deviation in these findings strongly suggestive of the occurrence of a pathological lesion past or present.

Holsti examined twenty-two hearts from cases of sudden death and found capillaries in the valve "roots" (*Wurzel*) of eight cases distributed as follows: eight in the mitral, six in the tricuspid and two in the aortic. The author, however, speaks of finding myocardium in the "roots" of many of his cases which obviously makes his definition of "root" different from our conception of what constitutes *Ring*. Furthermore, in eleven of the hearts, the author found definite evidence of valvulitis and in three cases marked vascularization of the "root" and valves as far as the tip. For these reasons it is impossible to compare statistically Holsti's findings with ours in respect to *Ring* vascularization.

While on this point it should be mentioned that a number of the hearts examined in our series showed inconspicuous scatterings of inflammatory cells. Often this was found in cases in which some form of sepsis had occurred during life. We did not mention these exudates in connection with our description of normal valves because they seemed obviously to be part of some pathological process, however slight.

#### NODULI ALBINI AND CONGENITAL VALVE HEMATOMATA

For completeness, mention may be made of the so-called Noduli Albini and of valve hematomata. According to Tandler, Cruveilhier was the first to describe the former. These consist of tiny jelly-like thickenings of the auriculoventricular valve substance and are to be found in the region of the insertions of the chordae tendinae of the first order. They are seen most frequently in the newborn and represent slight exaggerations of the myxomatous tip referred to in our previous sections.

The congenital valve hematomata consist of endothelial-lined spaces containing blood and are frequently found on the auricular aspect of the auriculoventricular cusps. These structures are seen only during the first year of postnatal life (Tandler). A number of

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myocardium in the auriculoventricular valves. A simple but comprehensive classification of the various valve layers has been given. In designing this classification there has been borne in mind the advisability of using a uniform nomenclature and of reducing the variations in valve structure to as few common denominators as possible. Attention has been drawn to the possible bearing which the various differences in valve structures may have on the development of pathological processes which occur at these sites. An indication has also been given of the evolutionary and involutionary postnatal changes which take place in the cusps.

The main purpose of the work here reported is to serve as a base line for further studies on disease of heart valves, because it is realized that an intimate knowledge of the structure of the valve cusps, together with their normal variations and age-period changes, is essential for the recognition and understanding of pathological processes.

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## DESCRIPTION OF PLATES

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### PLATE 102

- FIG. 1. *Right Aortic Cusp Section*. A, fibrosa; B, ring spongiosa; C, Annulus; D, subvalvular annulus; E, ventricularis; F, aorta; G, pulmonary conus; H, interventricular septum; I, arterialis; J, sinus pocket; K, pericardial wedge.
- FIG. 2. *Aortic Cusp Approaching the Commissure* showing changes in the arterio-annulus relations. A, aorta; B, annulus; C, pericardium; D, sinus pocket; E, aortic cusp; F, arterio-annulus junction.
- FIG. 3. *Aortic Cusp in the Immediate Vicinity of the Commissures* showing further changes in arterio-annulus relations. A, aorta; B, annulus; C, pericardium; D, aortic cusp; E, subcommissural elastica.
- FIG. 4. *Left Aortic Cusp Section*. A, left coronary artery; B, left ventricle; C, aorta; D, annulus; E, sinus pocket; F, aortic cusp; G, free visceral pericardium.

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PLATE 103

FIG. 5. *Posterior Aortic Cusp Section.* A, ring spongiosa; B, arterialis; C, sinus pocket; D, aorta; E, left auricular myocardium; F, left auricular endocardium; G, annulus; H, mitral-aortic intervalvular fibrosa; I, pericardial wedge; J, ventricularis.

FIG. 6. *Anterior Mitral and Posterior Aortic Valve Section.* For explanation of arrow "A" and arrow "B," see text. C, mitral-aortic intervalvular fibrosa; D, ring spongiosa; E, valve spongiosa; F, aortic valve fibrosa; G, mitral valve fibrosa; H, aorta; I, pericardial wedge; J, myocardial wedge (left auricle); K, left auricular endocardium; L, chorda tendinea.

FIG. 7. *Nodulus Arantii on aortic cusp.* A, nodulus arantii; B, aorta; C, ventricularis elastica; D, ring spongiosa; E, sinus pocket; F, pericardial wedge.

FIG. 8. *Posterior Mitral Valve Section.* A, spongiosa; B, fibrosa; C, posterior mitral valve pocket; D, pericardial wedge; E, auricular myocardial wedge; F, left auricular endocardium; G, left ventricle.



PLATE 104

- FIG. 9. *Cross-section of a Semilunar Cusp (High Power)*. A, ventricularis with its prominent elastica; B, spongiosa; C, fibrosa; D, arterialis.
- FIG. 10. *Cross-section of an Auriculoventricular Cusp (High Power)*. A, auricularis; B, spongiosa (inconspicuous); C, fibrosa; D, ventricularis.
- FIG. 11. *Left Pulmonary Cusp Section*. Note double myocardial crest made up of A, right ventricle, and B, left ventricle. C, pulmonary artery; D, pericardium; E, pulmonary cusp.
- FIG. 12. *Anterior Pulmonary Cusp Section*. A, pericardium; B, pulmonary artery; C, sinus pocket; D, ring spongiosa; E, pulmonary cusp; F, myocardium of pulmonary conus.

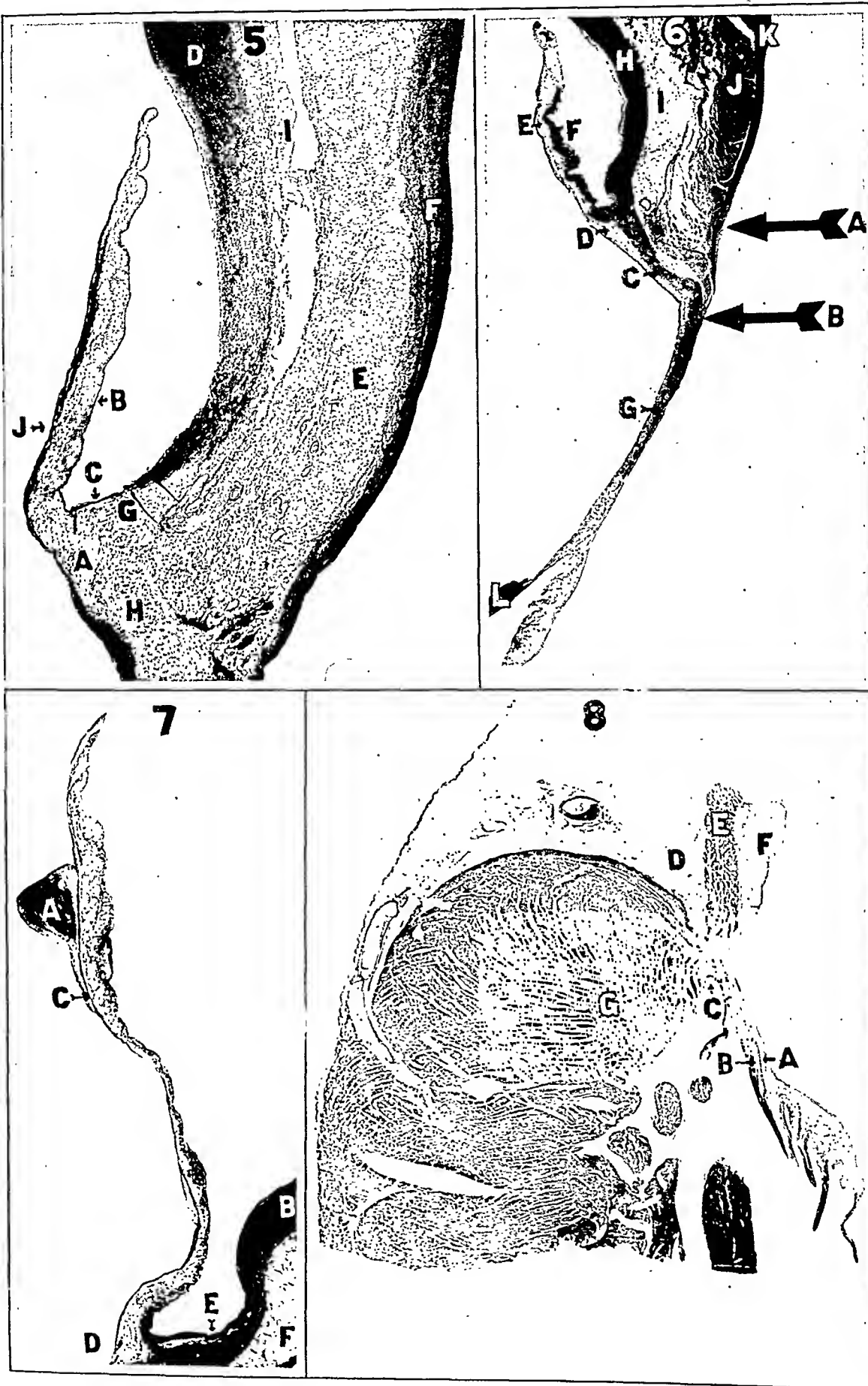


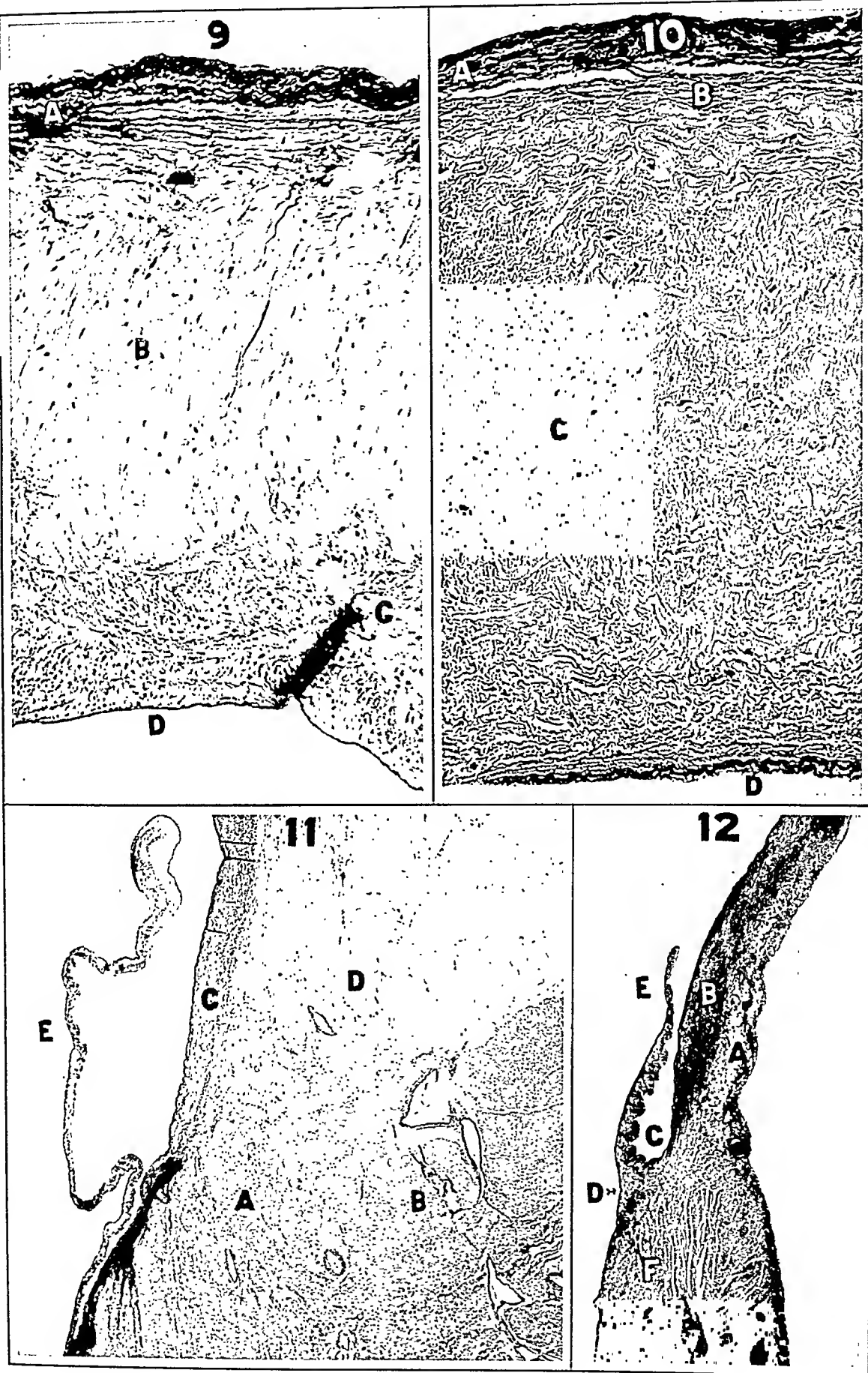
PLATE 105

FIG. 13. *Right Pulmonary Cusp Section*. A, pericardium; B, pulmonary artery; C, sinus pocket; D, valve cusp; E, ventricular myocardium.

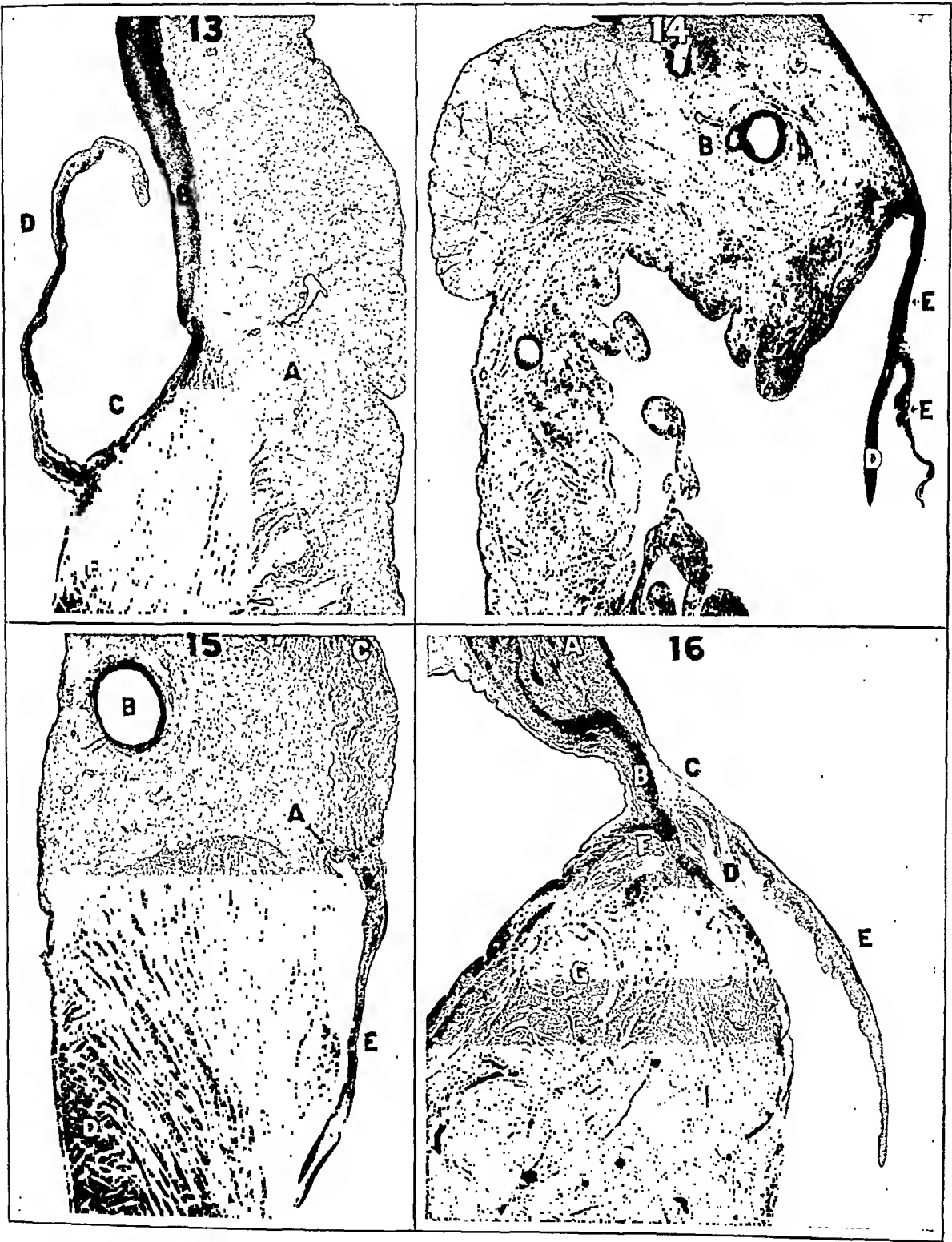
FIG. 14. *Anterior Tricuspid Valve Section*. A, trough-shaped myocardium of right ventricle; B, pericardial wedge with right circumflex coronary artery; C, right auricle; D, chorda tendinea; E, anterior tricuspid valve section; F, annulus.

FIG. 15. *Posterior Tricuspid Valve Section*. A, neck-like annulus; B, right coronary artery embedded in pericardium; C, right auricle; D, right ventricle; E, tricuspid leaflet.

FIG. 16. *Tricuspid Valve Section (septal flap)*. A, right auricular myocardial wedge; B, septum fibrosum; C, tricuspid valve ring; D, chorda tendinea insertion; E, septal flap; F, bundle of His; G, interventricular septum.









was enlarged three to four times its normal size. The lungs were but slightly involved, and rarely had a discrete lesion which would suggest tubercle formation. The bone marrow showed no gross evidence of tubercle formation, but it was always firm and congested. None of the other organs gave any evidence of involvement on gross examination.

A study of the histopathology of the various organs of these animals showed a number of interesting things. On the whole, all of the animals presented the same type of reaction in the various tissues, the only variations being in degree and not in absolute difference. Because of such close similarity in distribution of lesions and type of reaction it is deemed unnecessary to give protocols on each animal. The histological description will deal with the changes observed in the group as a whole.

The lungs had numerous areas in which there was considerable thickening of the alveolar walls. This thickening was due largely to an accumulation of mononuclear leukocytes. There was an occasional lymphocyte, and in some of the areas a few neutrophils were present. In these lesions tubercle bacilli were very scarce. The adjacent alveoli were often filled with an exudate similar to that present in the alveolar walls. On two occasions discrete lesions heavily infiltrated with neutrophils and showing evidence of early caseation were found. Both of these lesions contained large numbers of tubercle bacilli. On numerous occasions typical mononuclear tubercles were observed within the veins. In such lesions tubercle bacilli were scarce, but could be demonstrated.

If a considerable amount of exudate was present in the alveolar spaces, giant cells of the type usually seen in tuberculosis could be found free within the alveolar spaces. On rare occasions a few tubercle bacilli could be demonstrated in these structures. Bacilli were always scarce in the exudate even when it was abundant. The cellular content consisted almost entirely of mononuclear leukocytes.

The heart muscle occasionally showed small collections of lymphocytes or of mononuclear leukocytes. No tubercle bacilli could be demonstrated.

No evidence of inflammatory reaction was seen in sections of the gastro-intestinal tract. Very rarely one found tubercle bacilli within single mononuclear leukocytes in the lymphoid tissue of the gut.

## AVIAN TUBERCULOSIS IN NORMAL AND VACCINATED RABBITS \*

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The purpose of this report is to set forth the differences in the histopathology and in the leukocytic response in normal rabbits injected intravenously with the avian tubercle bacillus, and in rabbits which have been given some degree of protection by a previous subcutaneous inoculation of living tubercle bacilli of the human type. As far as we know, there is no record of spontaneous avian tuberculosis in the rabbit. Subcutaneous inoculation of virulent avian tubercle bacilli, even in doses of 10 mg., results in the formation of an abscess at the site of inoculation, with no generalized disease developing. With the rupture of the abscess this local lesion heals and the animal fully recovers.

If, however, one injects 0.5 to 1 mg. of virulent avian tubercle bacilli intravenously, rabbits will consistently die within a month. This is common knowledge to all who have used this method. The form of tuberculosis produced is commonly known as the Yersin septicemic type. It is frequently stated that there is no tubercle formation in the tissues in such animals. Blood cultures, taken at various intervals after infection, usually reveal the presence of the bacillus in the circulation.

### AVIAN TUBERCULOSIS IN NORMAL RABBITS

The twenty normal rabbits used in this study were given an intravenous injection of 0.5 to 5 mg. of virulent avian tubercle bacilli and all died within a month.

The most striking features of the gross pathology were the marked enlargement of the spleen, and, at times, a moderate enlargement of the liver. Neither organ had gross tubercles, such as occur in rabbits inoculated with the bovine type of tubercle bacillus. The spleen

\* Received for publication May 30, 1931.

capillary accumulation of cells. Tubercle bacilli were present in large numbers.

One of the interesting findings in the liver was the presence of giant cells which had all of the characteristics of megakaryocytes. In some areas there would be three or more of these cells in a high power field. Judging from their frequency in sections from different parts of the organ, the livers of some of the animals must have contained a large number of these cells. They were not observed in sections of the liver in every animal.

The cytological reaction observed in the spleen in these rabbits was the most interesting observed in any of the organs. Engorgement of the organs with blood was always present. In the majority of instances the splenic corpuscles were very small. Many of the germinal centers could be identified only by locating the central arteriole. A marked increase of mononuclear leukocytes was a constant finding. In some instances this increase was diffuse throughout the organ. At other times the pulp was studded with mononuclear tubercles. Some of these tubercles, especially in animals living about four weeks, showed necrosis of the mononuclear leukocytes with slight to marked infiltration with neutrophils. Typical caseation was seldom seen, while early tuberculous abscesses were often present. Foreign body giant cells were rarely encountered. Tubercle bacilli were always abundant. The lesions presenting necrosis of the mononuclear leukocytes with neutrophilic infiltration contained the greatest number of bacilli.

Large giant cells, similar to those seen in the liver, were very commonly found, at times as many as eight to ten in a high power field. These cells were observed in the germinal centers, in the splenic pulp and free in the venous sinuses. Mitotic figures were occasionally seen in these cells. In rare instances neutrophils were seen within the cytoplasm of these giant cells, just as they were observed in the megakaryocytes in the bone marrow of the same rabbit.

The malpighian corpuscles of the spleen were so insignificant in the sections studied, as compared with the same structures in the normal rabbit, that it was deemed advisable to kill animals at different stages of the disease to study the changes that occurred in this organ. A complete report of these studies and their apparent significance will be given at a later date. The most significant finding was the

These animals commonly had diarrhea and we were surprised not to find tuberculous lesions.

The pancreas appeared normal in all sections studied.

The kidneys showed very little evidence of inflammatory reaction. Such foci as were found consisted of small collections of lymphocytes and an occasional mononuclear leukocyte. Bacilli could not be demonstrated. On rare occasions tubercle bacilli within a mononuclear leukocyte were observed in an otherwise normal glomerulus.

The adrenals usually had a few small collections of mononuclear leukocytes in the cortex. The medullary tissue always appeared normal. Bacilli were usually abundant in the lesions. A few foci composed largely of neutrophils were seen. Tubercle bacilli were most abundant in such areas.

No lesions or bacilli were demonstrated in the genital organs of either the male or female rabbits.

No sections of joint tissue were studied, as all joints appeared normal in the gross.

Lesions in the bone marrow were a constant finding, as evidenced by discrete collections of mononuclear leukocytes. In some of the rabbits the major portion of the marrow was composed of a diffuse infiltration of these cells. Necrosis of the mononuclear leukocytes, with varying degrees of infiltration with neutrophils, was often observed. Bacilli were easily demonstrated wherever the monocytic accumulation was found, and were present in large numbers in the more diffuse lesions. The hematopoietic tissue of the marrow was always hyperplastic even though extensive tuberculosis was present. Mitotic figures were easily found in leukopoietic and erythrocytic centers. Megakaryocytes were increased in number and mitoses were frequently found in cells of this type. These cells were often seen projecting into, and free within the capillaries of the marrow. A considerable number of megakaryocytes had neutrophils within their cytoplasm. Foreign body giant cells of the type seen in tuberculosis were very rare, even when the lesions were extensive.

The liver always showed extensive involvement. In some instances the volume of inflammatory reaction was greater than the volume of liver tissue. The reaction consisted largely of mononuclear leukocytes within the capillaries of the organ. Occasional mitotic figures were found in the mononuclear leukocytes. The parenchyma of the liver was often markedly compressed, due to the extensive intra-

The neutrophiles and mononuclear leukocytes were about equal in number and were more than 1,000 per cubic millimeter.

The leukocytic response in all the other animals was fairly constant in the differential percentages, but showed considerable variation in the total leukocytic counts. Twenty-four hours after the injection of the tubercle bacilli a slight rise in neutrophiles occurred. At the same time, or more often at the end of forty-eight hours, an initial small rise in mononuclear leukocytes could be demonstrated. This initial response on the part of the neutrophiles and mononuclears receded promptly to normal for the individual rabbit. At this stage no significant change in the lymphocyte count was manifest.

For a period of eight to ten days the most striking change in the leukocytes was a tendency for the neutrophiles to decrease in number and for a slight leukopenia to develop. After this stage of incubation a rapid alteration in the leukocytic formula occurred which persisted until death. The first outstanding change was a rapid rise in the number of mononuclear leukocytes. The peak of this rise was often reached within three or four days after its inception, and from this time on these cells did not recede to a normal level. Great daily fluctuations in counts were observed. During this stage one occasionally encountered mononuclear leukocytes undergoing mitosis. The cells were markedly vacuolated at times and it was not uncommon to find red blood cells within their cytoplasm.

Prior to the increase of circulating mononuclear leukocytes there was no significant change in the number of lymphocytes. Some of the animals showed a sharp decrease in the number of lymphocytes at the time of the main mononuclear response. From the date of the most marked increase of mononuclear leukocytes to the death of the animal, the number of circulating lymphocytes tended to decrease, although one observed a considerable fluctuation from day to day. In several rabbits these cells decreased to less than 500 per cubic millimeter, whereas the same rabbits had 5,000 to 8,000 lymphocytes per cubic millimeter before they were infected.

The neutrophilic response in these rabbits usually lagged behind that of the mononuclear leukocyte. In every rabbit the neutrophiles returned to a normal level within two or three days after the mononuclear rise. From this time on these cells tended to be above normal and they were generally the predominant cell type before the

tremendous hyperplasia that occurred in the germinal centers up to about fourteen days after the injection. Because of the large numbers of mitoses and of the extension of the germinal centers well beyond their normal borders into the splenic pulp, one would be led to suspect that a malignant condition existed, were it not known that this reaction was due to infection with the tubercle bacillus.

### *The Leukocytic Response*

The leukocytic response in these rabbits was determined by daily total and differential counts. The technique used was the same that we have adopted in the study of the human blood. Blood samples were obtained in all instances from a vein in the ear. Total counts were made from a 1:20 dilution of the blood, using the Levy counting chamber. Blood smears were stained with Wright's blood stain. We recognize that in rabbits' blood it is difficult to distinguish clearly between young mononuclears or monocytes and large lymphocytes with this method of staining. However, as the disease process advanced, the change in the percentage of the different leukocytic types was so marked that one could gain a fairly accurate idea of the pathological process as a whole with this technique. We have found this procedure to be simpler and less time-consuming than the supravital method of staining.

To illustrate the changes in the circulating leukocytes during the evolution of the disease, charts R-B1., and R-8 have been included. These show the variation that may occur in different animals with different doses of bacilli. We found it impossible to get a satisfactory average leukocytic picture in normal rabbits, so we adopted the procedure of using each animal as its own control by making daily counts for a week or longer before the injection of tubercle bacilli was given.

Only the main features in the change of the leukocytic picture will be discussed as all the fluctuations are not considered significant. The variations are largely individual changes that one would expect to occur in different animals. Two rabbits in the series died in fourteen days. Both of these animals were given an injection of 5 mg. of avian tubercle bacilli and each developed a marked leukopenia with the greatest decrease occurring in the lymphocytes. At the time of death the lymphocytes were less than 1,000 per cubic millimeter.

tubercle bacilli. The non-vaccinated animals all died within 24 days. In the vaccinated group one animal died on the 149th day, one on the 140th day, one on the 122d day, and one on the 7th day.

The last rabbit succumbed so quickly that we at first thought it died of some intercurrent infection. At autopsy the spleen was found markedly enlarged, the liver was slightly enlarged, and there was no evidence of lobar pneumonia. There was no evidence of snuffles. No typical tubercles were seen in any organ on gross inspection. Joints, kidneys, testes and epididymes appeared normal. The bone marrow of the femur was firm and red. At the site of the inoculation with human bacilli there was a small scar. The gross findings were identical with those of normal rabbits inoculated with the avian tubercle bacillus, except that no normal rabbit had ever shown such marked enlargement of the spleen and liver at such an early date. The absence of old tubercles in any of the organs is of importance in consideration of the lesions the other rabbits in the group showed.

Histological studies of the tissues of this rabbit showed extensive tubercle formation in the spleen, liver and bone marrow. Pulmonary lesions similar to those described above for the normal rabbit group were present. A finding of some surprise was the extensive tubercle formation and the scarcity of tubercle bacilli. Bacilli were found but they were very infrequent. Foreign body giant cells were much more numerous than in the lesions of the non-vaccinated rabbits.

Cultures were not made of the lesions of this animal, but from the extensive pathology in the liver, spleen and bone marrow we feel convinced that death was due to the avian tubercle injection given a week before.

The pathology of the other three rabbits in the group was so uniform that we will describe them as a group. All of the rabbits before death became so emaciated that they could not stand. Their appetites remained good and they would eat food if it were placed where they could reach it in the prone position.

The gross pathology showed a striking contrast to that seen in the non-vaccinated animals. The spleen was of normal size and of a dark brown color. Rare firm, grayish tubercles of millet-seed size were present. The liver was of normal size. There were numerous scars and occasional minute tubercles were present beneath the capsule and in the liver substance. The lungs in one rabbit were normal. In each of the other two there were found two or more foci

animals succumbed to the infection. Here again considerable daily fluctuation was observed.

There were marked variations in the numbers of basophiles and of eosinophiles during the progress of the disease, but toward the end of the process these cells were scarce in the circulating blood.

The total number of leukocytes showed considerable variation from day to day. A mild leukocytosis was observed as the disease progressed and in some of the animals there occurred a marked leukocytosis before death.

Red blood cell counts were not done as these cells take no part in the production of the tuberculous lesions. It was noted, however, that there was marked anemia and at times nucleated red blood cells were numerous in the blood films.

Blood platelet counts were not done.

#### AVIAN TUBERCULOSIS IN VACCINATED RABBITS

We turn now to the rabbits which were partially protected. There were but four rabbits in this group. While the group is small the findings differ so markedly from those in the non-vaccinated group that we consider them very significant. As daily blood counts were done on this group and on a group of eight normal rabbits inoculated with the same dose of avian tubercle bacilli on the same date, we felt that the work entailed prevented a larger group from being studied. We felt also that intensive work done on a few animals would give more significant data than less work on a larger group.

These four rabbits were originally used in another study. A subcutaneous inoculation of 5 mg. of human tubercle bacilli, H-37, had been given six months before they were used in the present experiment. Each animal had developed a local abscess at the site of inoculation. These abscesses had ruptured and the local lesions had healed. At the time of inoculation with the avian tubercle bacillus one rabbit showed a small lump about the size of a pea at the site of the previous inoculation. The other rabbits showed no local lesions. These findings were confirmed later at autopsy. When inoculated with the avian bacillus the rabbits were in excellent physical condition and their blood counts were normal.

The group of twelve rabbits, eight normal and four vaccinated, were given an intravenous inoculation of 1 mg. of virulent avian



those that were present showed slight caseation, foreign body cells, considerable lymphocytic infiltration and fibrosis. Isolated foreign body giant cells were commonly seen. Bacilli were found with difficulty.

The major portion of the liver tissue appeared normal. There were occasional tubercles with central caseous areas, giant cells, and considerable lymphoid infiltration in the periphery. Considerable fibrosis was present in most of the lesions. There were many small collections of lymphoid cells in the parenchyma. These evidently were the remains of a once active tuberculous focus. Microscopic abscesses were occasionally seen. Scars, composed of fibrous tissue infiltrated with lymphocytes, were present. These probably represented healed tuberculous lesions. Many isolated foreign body giant cells were seen. Bacilli were not found in the scarred areas or in the collections of lymphoid cells. They were found after long search in some of the caseous tubercles, and with ease in the abscesses.

The kidneys showed large, caseating tuberculous lesions, most of which were pyramidal in shape, extending from the cortex into the pyramids. Giant cells were rare. Occasional scars were present. In some lesions, where neutrophils were abundant, bacilli occurred in large numbers. In the old caseous lesions bacilli were very scarce and in the scars bacilli could not be demonstrated.

Sections from the adrenals and the gastro-intestinal tract revealed no lesions. Sections from the lung lesions showed old caseous tubercles with some calcification and with rare bacilli present. In the lymph nodes there were a few small mononuclear tubercles. The testes and epididymes had caseous foci. Sections of tissue from around the joints revealed abscesses with caseating peripheries. Bacilli were easily found.

### *The Leukocytic Response*

The leukocytic response of these animals was quite in contrast to the non-vaccinated group. Chart R-I-H-A, of the rabbit dying in 7 days, and Chart R-4-H-A of the one dying in 144 days are appended to give a graphic picture of what occurred. All of the animals had normal leukocyte counts prior to the inoculation of the avian bacillus, showing that they had recovered from their infection with the human bacillus. Chart R-I-H-A shows that the leukocytic picture

of typical tuberculosis. These areas appeared like conglomerate tubercles. The gastro-intestinal tract was negative throughout. There was extensive bilateral renal tuberculosis. The adrenals appeared normal. Two of the rabbits showed circumscribed tuberculous lesions in the testes and epididymes. The bone marrow was congested and friable but no gross tubercles were seen. Each of the rabbits showed multiple tuberculous joints, with abscess development of considerable extent at some of the joints, notably knees, hips and elbows. Some of these lesions extended for a considerable distance along the fascial planes of the muscles. At the site of inoculation of the human tubercle bacillus two rabbits showed small scars. The third rabbit showed a small caseous lesion. This lesion had enlarged and had discharged its contents during the course of the avian infection.

Smears from the various tuberculous lesions showed a few to many acid-fast bacilli. No other bacteria were seen. Cultures from the abscessing joints and from the kidneys gave a pure growth of acid-fast bacilli of the avian type. No human type of tubercle bacilli were obtained in these cultures. From the bacteriological point of view it would seem that, although the lesions differed in gross appearance from those seen in the unvaccinated animals, they were caused by the avian tubercle bacillus.

Smears made from the residual caseous lesion of the human tubercle bacillus infection present in one of the rabbits revealed no acid-fast organisms and culture was negative.

Histological studies of the tissues of the three animals revealed typical tubercle formation with caseation and numerous giant cells. There was calcification in many of the lesions. The bone marrow revealed an occasional foreign body giant cell and a rare tubercle composed largely of scar tissue and of lymphoid cells. There was marked atrophy of the fat tissue. The hematopoietic tissue was moderately increased. Mitotic figures were easily found, especially in the leukopoietic centers. Megakaryocytes were not greatly increased in number. Many of these cells had neutrophils within their cytoplasm. Bacilli could not be demonstrated in the marrow lesions.

In the splenic pulp there was a very heavy deposit of brownish pigment. The germinal centers appeared smaller than normal but mitotic figures were occasionally seen. Tubercles were scarce and

our infective agent, we do not feel that the histopathology which it induced is significantly different from that produced by other types of tubercle bacilli.

There is no question that there may be a difference of degree in the histopathology caused by tubercle bacilli of the avian, bovine or human type, but there is no specific difference. One can find a great variation in the histopathology caused by bovine tubercle bacilli of varying degrees of virulence, or caused by a single strain of bovine tubercle bacillus given in different doses. It is quite probable that there is some difference in the chemical composition of the various types of tubercle bacilli. It is also probable that the chemical composition of a very virulent bovine tubercle bacillus differs somewhat from that of a bovine tubercle bacillus of low virulence. Specific biochemical reactions must occur in the tuberculous lesions but, at present, the histopathology seen cannot be interpreted on the underlying specific biochemical changes. The writer is of the opinion that if one takes into account the histopathological picture present in all the tissues it is impossible to state with certainty the type of tubercle bacillus that has been the infective agent.

There may also be a difference in the gross pathology of rabbits infected with the avian, bovine or human type of the tubercle bacillus. One cannot, however, regard this difference as specific. Our study shows that by altering the condition of the rabbit, namely by vaccinating it, the gross pathology caused by the avian tubercle bacillus differs markedly from the gross pathology observed in the non-vaccinated rabbit.

In all articles with which the author is acquainted, evidence of resistance in vaccinated animals is measured by the length of life, as compared to non-vaccinated controls, and the presence and the extent of gross tuberculous lesions. We wish to call attention to the fact that there is quite a striking difference in the histopathology in vaccinated and non-vaccinated animals. In the first place, tubercle bacilli are uniformly much more numerous in the lesions of non-vaccinated animals than in the vaccinated. Typical tubercles with caseous centers, giant cells, lymphoid infiltration and fibrosis are commonly seen in vaccinated animals, whereas such structures are not found in non-vaccinated animals when they die in from three to four weeks. Scars, often of considerable size, are found in the tissues of vaccinated animals, whereas they are not found in the non-

changed rapidly to a markedly septic type, and the animal quickly succumbed.

Chart R-4-H-A is typical of the three remaining rabbits. The mononuclear leukocytes played a prominent, but not so spectacular a part, as in the non-vaccinated animals. Considerable daily fluctuations occurred. For a considerable period before death these cells became as numerous or more numerous than the lymphocytes.

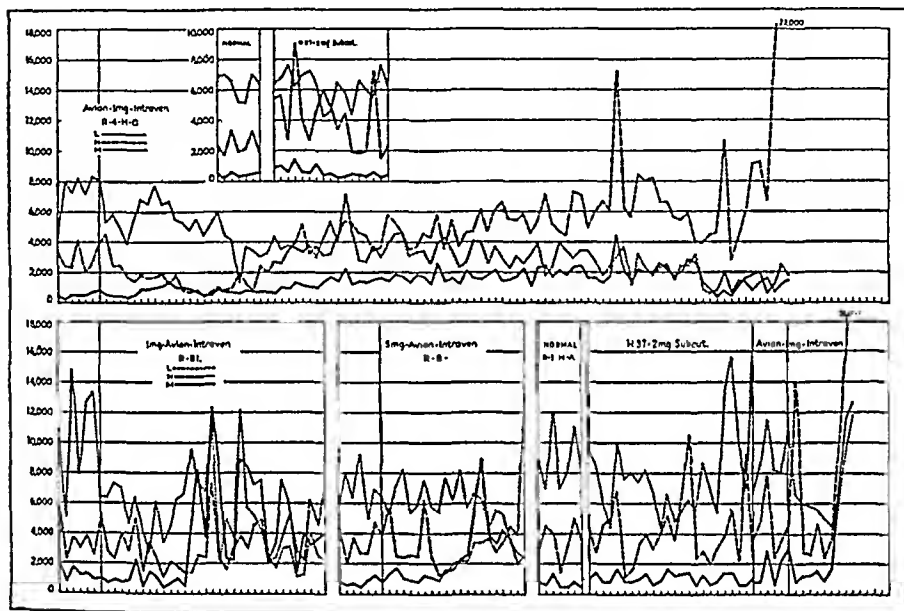


CHART I

The most striking feature of the chart is the gradual change of the leukocytic picture to the septic type which remained, after it was once established, until the animal died. The neutrophils also showed wide daily fluctuations. In each of the animals in this group the septic leukocytic reaction became definitely established weeks before the death of the animal.

The lymphocytic picture was just the reverse of the neutrophils. There occurred a gradual decrease of these cells to a low level where they stayed, except for some daily fluctuation.

### DISCUSSION

We have given above the pathology and the leukocytic reaction in normal and in vaccinated rabbits intravenously infected with virulent avian tubercle bacilli. While we used the avian tubercle bacillus as

The striking difference in the leukocytic reaction of the two groups can be noted in the leukocytic graphs. The most striking feature is that the leukocytic picture gradually changed in the vaccinated rabbits to a septic reaction and remained so until the death of the animal. We do not attempt to explain this change. It is of interest, however, that in progressive cases of human tuberculosis the leukocytic reaction is very similar to that seen in the vaccinated rabbits. We are of the opinion that this septic reaction is in response to the damaged tissue in the tuberculous lesions and is due to unmixed tuberculous infection.

The vaccinated animal which died in 7 days was of interest. We have not had a normal animal succumb so quickly to a tuberculous infection, even when massive injections have been made. With such extensive pathology the scarcity of demonstrable tubercle bacilli was wholly unexpected. It seems probable that the previous vaccination with the human tubercle bacillus had enabled this animal to destroy the majority of the tubercle bacilli injected quickly, but the increased resistance was insufficient to render harmless the products of the destroyed bacilli. The flooding of the body with the toxic substances of the destroyed bacilli was probably so great that the animal was unable to render them harmless before death ensued. Even the quick and extensive response on the part of the leukocytes failed to stem the tide. One might postulate that the degree of resistance which the vaccination had produced was the prime factor in the sudden death of the animal. Whether the degree of resistance in this animal was greater or less than in the other animals in the group is an open question.

A renewal of interest in the relationship of Hodgkin's disease to tuberculosis has recently been created through the work of L'Esperance.<sup>4</sup> This article is of interest in relation to the reports of L'Esperance, as it presents the acute and chronic types of pathological reaction in the rabbit to the avian tubercle bacillus, which L'Esperance is inclined to believe is the etiological agent of Hodgkin's disease. There are certain phases of the pathology which simulate Hodgkin's disease. The significance of this observation will be reported in another article.

vaccinated. Organs which were very heavily involved in non-vaccinated animals gave little evidence of involvement or much permanent impairment in vaccinated animals. Organs which were very slightly involved in normal animals became, in the vaccinated, the seat of serious involvement, with some evidence of resistance as shown by the presence of fibrosis, calcification and giant cell formation. This resistance, however, was insufficient to overcome the massive damage done to such tissues. These findings are all the more significant, because in our experiments we have used an intravenous dosage of virulent avian tubercle bacilli that has in every instance killed normal rabbits within a month.

The findings given above help one to understand better the lesions one sees in chronic tuberculosis in animals and in human beings. In unreported experiments on prophylactic vaccination against tuberculosis in laboratory animals, we have commonly seen a marked difference in the histopathology between vaccinated animals and controls, but we had not appreciated that this difference signified the increased resistance on the part of the vaccinated animals. Failure to find tubercle bacilli in lesions with giant cells, lymphoid infiltration and fibrosis was attributed to non-stainable bacilli. We now believe that in the majority of such lesions the bacilli have been destroyed. This same interpretation would apply logically to the usual text book "epithelioid" tubercle in human tuberculosis and would explain the scarcity of tubercle bacilli in these lesions. In other words, tubercles with caseous centers and giant cells represent an increased resistance on the part of the host. If the pathology of tuberculosis were limited to this picture, one would not find cavitation and extensive involvement of tissues which lead to death. Such tubercles indicate a regression rather than a progression of the disease.

A considerable revival of interest has been created in the leukocyte reaction in tuberculosis of late. The pioneer work in this country, with regard to the new interpretation of the leukocytic reaction, is that of Sabin and her coworkers.<sup>1</sup> Their interpretation is based largely on the textbook description of the pathology of tuberculosis, in that they relegate the neutrophile to a place of no importance, so far as the tuberculous process *per se* is concerned. More recently we have offered <sup>2</sup> an interpretation of the leukocytic reaction based on a somewhat different interpretation of the pathology of tuberculosis.<sup>3</sup>

## DESCRIPTION OF PLATES

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### PLATE 106

FIG. 1. Lung from non-vaccinated rabbit. Numerous small tubercles. Death in 24 days.  $\times 50$ .

FIG. 2. Lung from vaccinated rabbit infected with avian tubercle bacilli. Death in 140 days.  $\times 10$ .

## SUMMARY AND CONCLUSIONS

1. There is a significant difference in the gross pathology and in the histopathology of non-vaccinated and vaccinated rabbits intravenously inoculated with virulent avian tubercle bacilli.

2. The text-book description of the tubercle — “a collection of epithelioid cells set in a reticulum with a giant cell in the center and a tendency to undergo caseation” — represents a retrogressive, not a progressive phase of the pathology of tuberculosis.

3. There is a striking difference in the leukocytic reaction of non-vaccinated and vaccinated rabbits intravenously infected with virulent avian tubercle bacilli.

4. The leukocytic response in the vaccinated rabbits simulates very closely the leukocytic reaction we have observed in human beings who have progressive tuberculosis. This reaction is not specific for tuberculosis, but is caused by unmixed tubercle bacillus infection.

5. The megakaryocyte plays an important rôle in acute avian tuberculosis in the rabbit. What the real significance of the participation of the megakaryocyte in acute tuberculosis is, cannot be stated at present.

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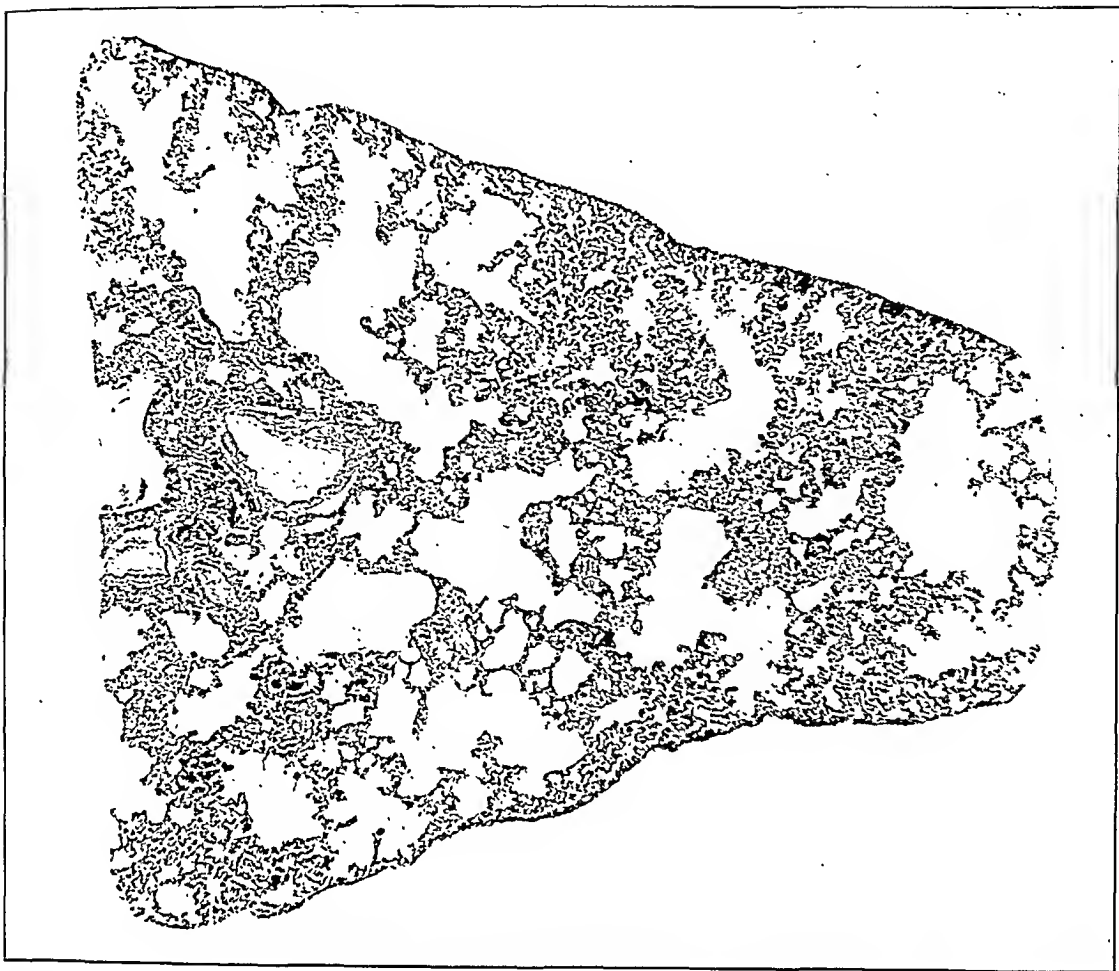


PLATE 107

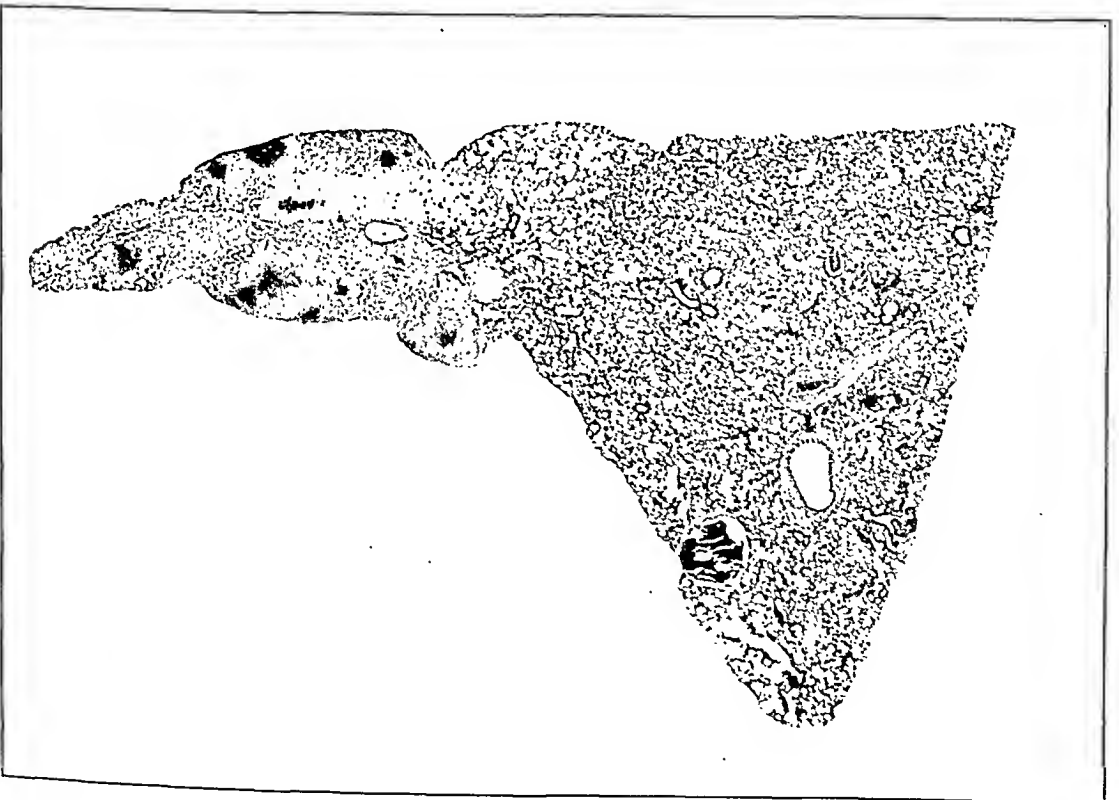
FIG. 3. Lung from non-vaccinated rabbit. Tubercle within vein.  $\times 600$ .

FIG. 4. Spleen from vaccinated rabbit. Avian tubercle bacillus infection.  
Death in 140 days.  $\times 10$ .

FIG. 5. Spleen from non-vaccinated rabbit. Death in 28 days.  $\times 10$ .



I



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Medlar

Avian Tuberculosis in Rabbits

PLATE 108

FIG. 6. Spleen from non-vaccinated rabbit. Enlarged area of Fig. 5. Tubercle with neutrophile infiltration in center.  $\times 500$ .

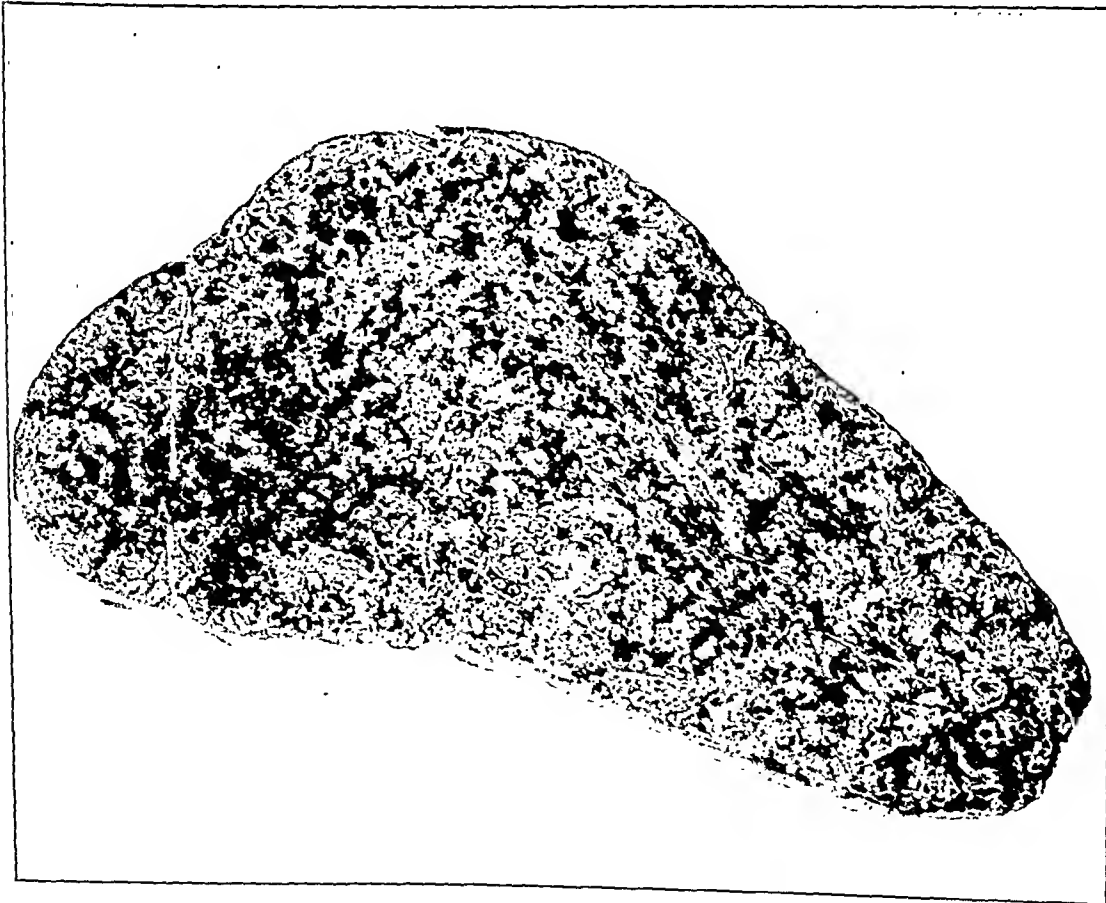
FIG. 7. Kidney from vaccinated rabbit. Death in 140 days.  $\times 10$ .



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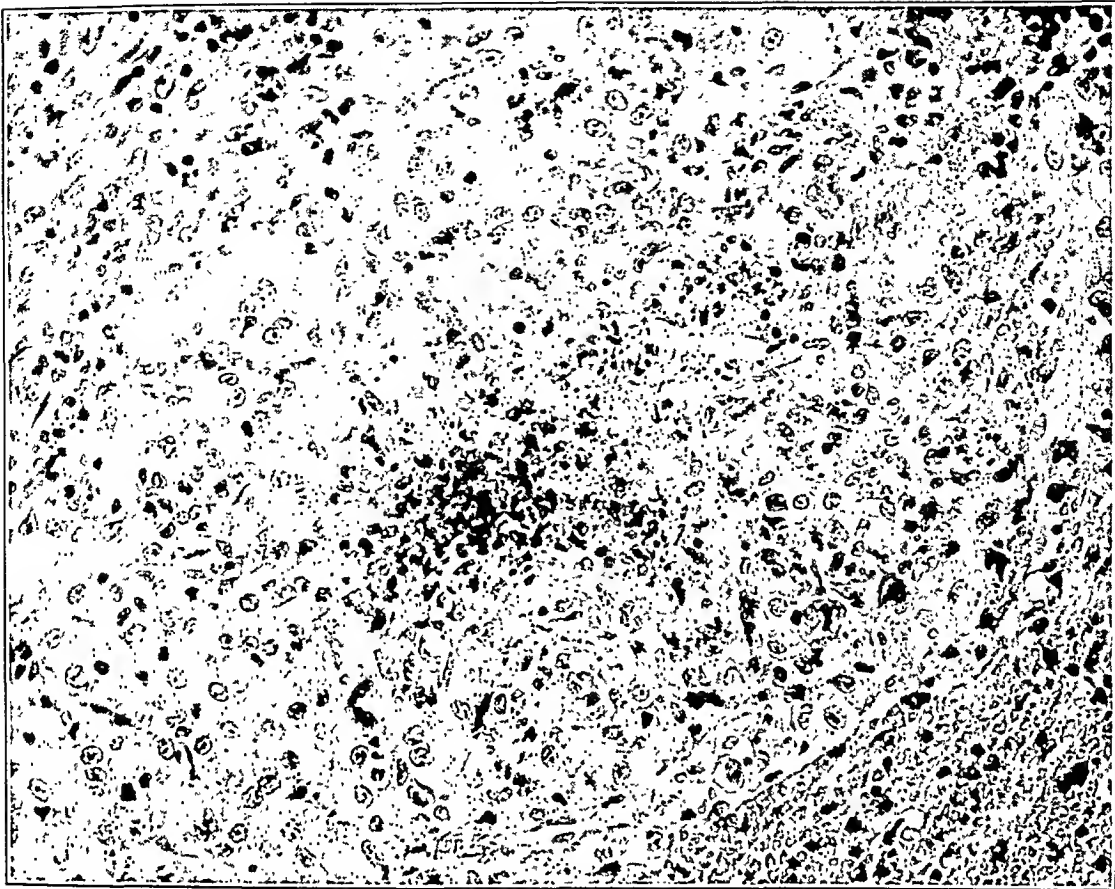
Medlar

Avian Tuberculosis in Rabbits

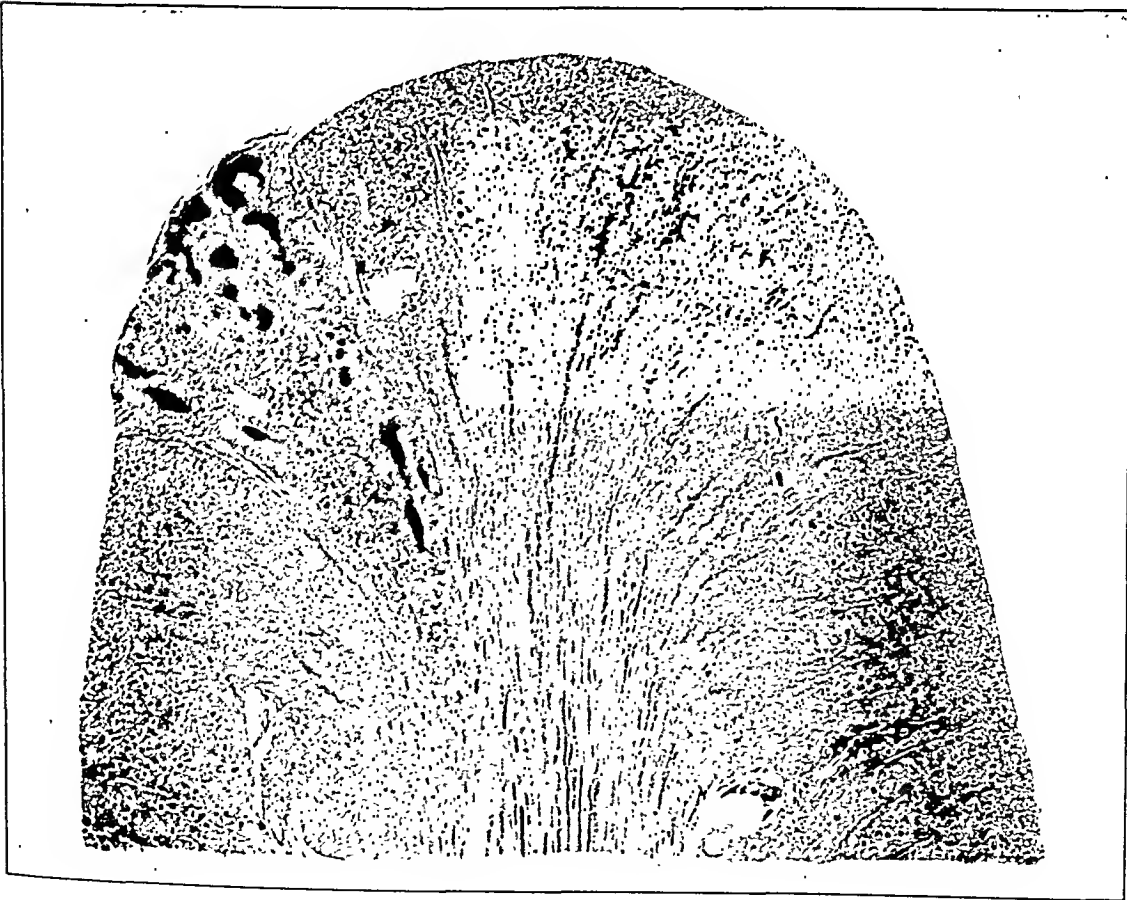
PLATE 109

FIG. 8. Liver from non-vaccinated rabbit. Tubercle in distended sinusoid.  
× 800.

FIG. 9. Liver from vaccinated rabbit. Large scar. × 10.



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Medlar

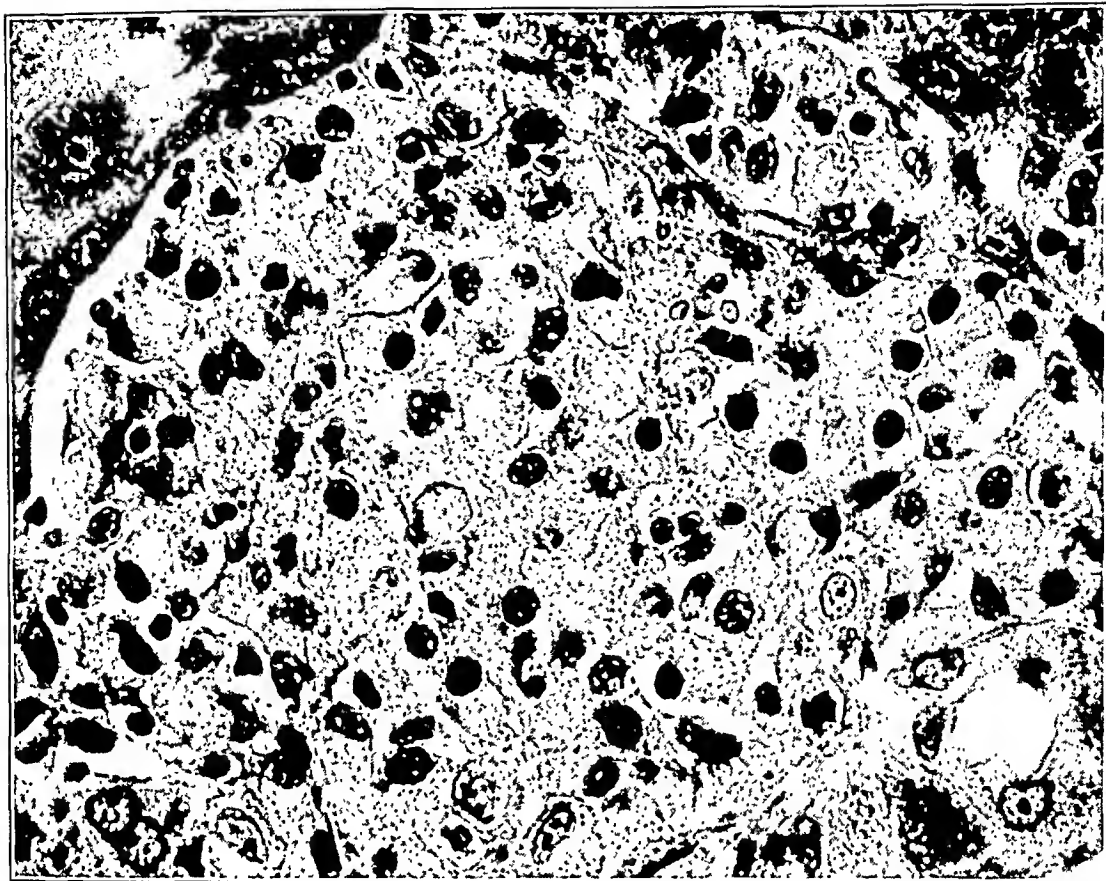
Avian Tuberculosis in Rabbits

PLATE 110

FIG. 10. Bone marrow from non-vaccinated rabbit. Lower megakaryocyte entering small vein to left.  $\times 800$ .

FIG. 11. Same section as Fig. 10. Two megakaryocytes in mitosis.  $\times 800$ .

FIG. 12. Marrow from non-vaccinated rabbit. This marrow showed extensive tuberculosis and large numbers of bacilli. Note bacilli, mononuclear leukocyte infiltration and some neutrophile infiltration.  $\times 800$ .



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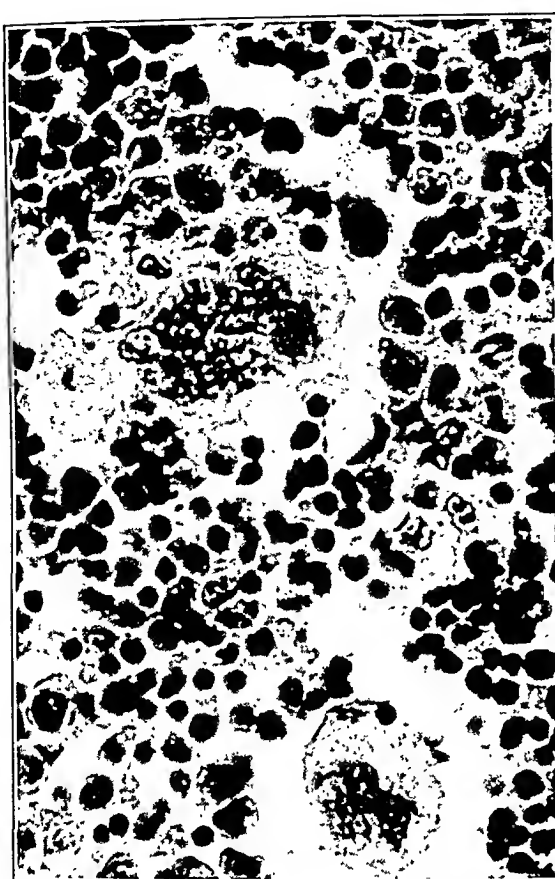
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Medlar

Avian Tuberculosis in Rabbits



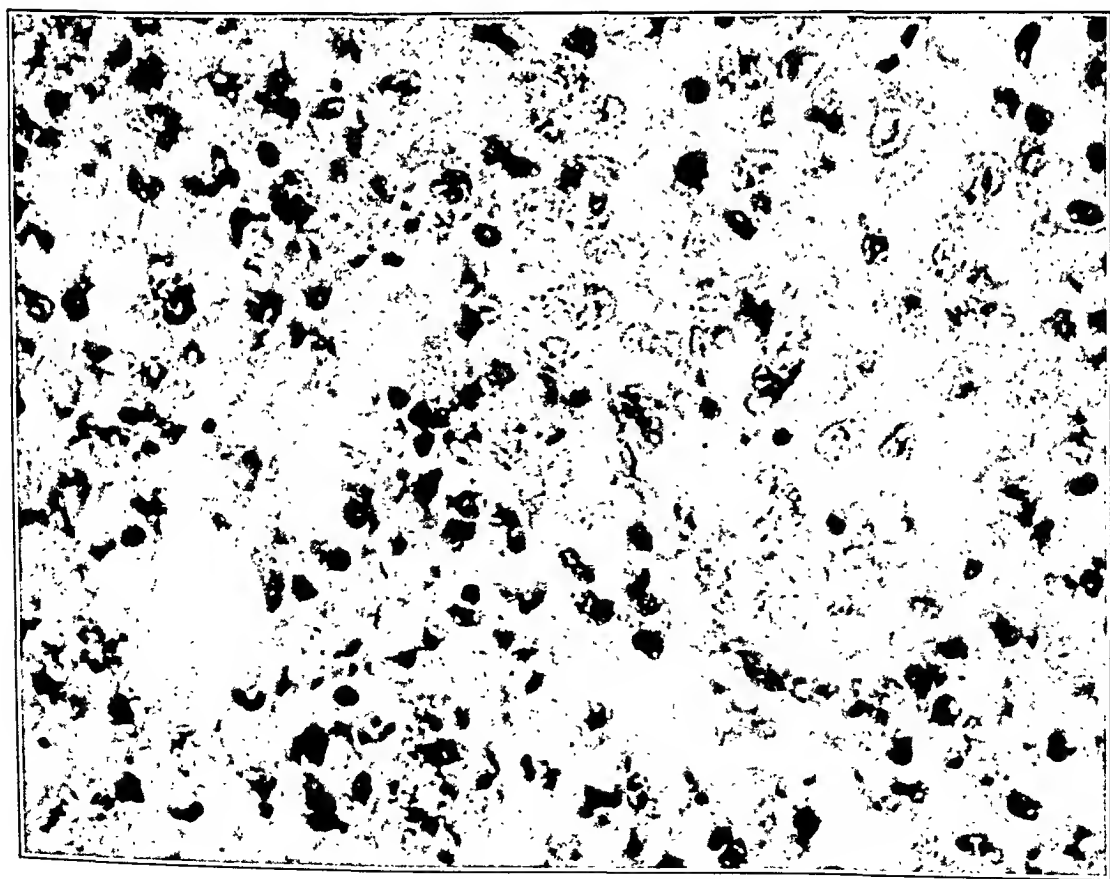




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Medlar

Avian Tuberculosis in Rabbits

In this report we will present an interpretation of certain lesions resembling Hodgkin's disease which we have observed in our studies of the tissues in experimental tuberculosis, as well as similar lesions in a human being who died from extensive pulmonary tuberculosis.

Our first observations were on rabbits that had been infected intravenously with virulent avian tubercle bacilli. These animals died in from two to four weeks. The gross and microscopic pathology, as a whole, is reported in another article so that in this report we will mention only lesions simulating Hodgkin's disease. These animals did not give the gross pathology of Hodgkin's disease. Microscopic examination of the various tissues showed a few to many giant cells which were indistinguishable from the giant cells seen in Hodgkin's lesions. Such lesions were found in the lung, liver and spleen. The bone marrow in these animals was markedly hyperplastic and showed a marked increase of megakaryocytes. We were able to find the megakaryocytes in the process of entering the circulation and present within the blood sinuses of the marrow. These findings led us to the conclusion that the giant cells observed in the tissues were megakaryocytes.

In the hyperplastic marrows complex mitotic figures in the megakaryocytes were occasionally encountered. These, also, were found rarely in the spleen and liver. Such figures resembled very closely the complex mitotic figures occasionally seen in the giant cells of Hodgkin's lesions.

Mitoses were more commonly encountered in a cell type in the marrow that was much smaller than the megakaryocyte but was somewhat larger than the myelocytes in the same section. It appeared that these smaller cells were the parent cells or the pre-megakaryocytes. The significance of this observation will be dealt with in a subsequent report on Hodgkin's disease.

These lesions were much more numerous in some of the rabbits than in others. In animals that were given a smaller dosage of bacilli or were protected to a degree by vaccination with tubercle bacilli and lived for several months, not only was the type of the tuberculous lesion different, but we did not observe any lesions suggestive of Hodgkin's disease in the tissues. From this it became apparent that only at certain stages of acute avian tuberculosis in the rabbit did the megakaryocytes wander out of the marrow in sufficient numbers to enable their detection in numbers in the tissues.

## THE SIGNIFICANCE OF LESIONS RESEMBLING HODGKIN'S DISEASE IN TUBERCULOSIS \*

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Interest in the relationship between tuberculosis and Hodgkin's disease has been revived through the reports of L'Esperance.<sup>1</sup> According to these reports, L'Esperance is of the opinion that the tubercle bacilli which she obtained from certain cases of Pel-Ebstein disease were of the avian type. The deduction from her studies was that the avian tubercle bacillus probably has an etiological relationship to Hodgkin's disease.

Numerous investigators have reported on the association of tuberculosis and Hodgkin's disease. Ewing<sup>2</sup> states that "tuberculosis follows Hodgkin's disease like a shadow." While there have been numerous observations of the association of these two diseases no one has been able to reproduce in experimental animals the gross and histological pathology of Hodgkin's disease with the tubercle bacillus. Failure to reproduce Hodgkin's disease experimentally has led those who believe that the tubercle bacillus is the etiological factor to postulate that it is necessary to have a tubercle bacillus of a certain type and of a certain pathogenicity. To date this special type of organism has not been obtained.

Because of inconstancy of findings relative to the tubercle bacillus in Hodgkin's lesions other investigators have been led to search for a different infectious agent. The bacteriological studies of Bunting and Yates<sup>3</sup> revealed the common occurrence of a diphtheroid organism. With this organism they were unable to reproduce typical Hodgkin's disease experimentally. Other investigators have obtained still different bacteriological findings. Recently Haythorn<sup>4</sup> has obtained a culture of a monilia in a case of Hodgkin's disease. The varied bacteriological findings have led some who believe that Hodgkin's disease is of an infectious nature to the conclusion that the etiological agent has not been discovered.

\* Received for publication May 30, 1931.

## DISCUSSION

From the illustrations which accompany this article one can see the close similarity between the megakaryocytes in the acute tuberculous animals and the Sternberg giant cells which one finds in Hodgkin's lesions in human beings. The significance of this observation will be discussed in a future article on Hodgkin's disease.

It is of interest that in acute tuberculosis caused by different types of tubercle bacilli in different animals there is a marked response on the part of the megakaryocyte as well as of the mononuclear leucocyte and the neutrophile. It is commonly observed that there is a marked increase and abnormally large platelets in the circulation of animals and of man in extensive acute tuberculosis. It is not surprising, therefore, that there is a hyperplasia and a wandering out from the bone marrow of the megakaryocytes which are the cells from which platelets are derived. There can be no question that these cells do leave the marrow and that they wander about in the tissue. They have been observed free in an alveolar space in the lung. The part that the megakaryocyte and its platelets play in the acute tuberculous process is not known. Fibrin is abundant in these acute tuberculous lesions and it may be that the megakaryocyte and the platelets play a definite rôle in fibrin formation.

The increase of platelets and the presence of megakaryocytes in acute lesions is not peculiar to acute tuberculosis. Such a condition is often seen in acute lobar pneumonia. It does not appear to be specific for any infection and in all probability it is a general biological phenomenon which occurs in acute infectious and non-infectious injury to tissue. For instance, saponin, when injected into rabbits, will cause a marked emigration of megakaryocytes from the bone marrow into the tissues, the animals dying in four or five days. The ability to produce such a condition with a drug shows clearly that the response on the part of the megakaryocyte is not dependent upon a bacterial infection.

In none of the animals which we have studied has the gross pathology suggested Hodgkin's disease. This is true whether the disease was acute or chronic. In the human case cited in this article there was no gross pathology which suggested Hodgkin's disease. To date we have been unable to find any lesions in tuberculous tissues which would suggest Hodgkin's disease, except in the histopathology of

Our next observations were on rabbits, guinea pigs and calves which were inoculated with a virulent bovine tubercle bacillus. The strain of tubercle bacillus used in this study was a culture of B.C.G. (the strain of tubercle bacillus advocated by Calmette for prophylactic vaccination against tuberculosis), which had had its virulence greatly enhanced by growing in a favorable environment *in vitro*. The rabbits and calves were inoculated intravenously and the guinea pigs subcutaneously. All of the animals in this study died in from three to four weeks from extensive acute tuberculosis.

Since the three types of animals showed essentially the same type of lesions which resembled Hodgkin's disease it is unnecessary to describe them separately. The calves showed the most marked lesions. Numerous groups of megakaryocytes were found in tuberculous lymph nodes and in hemolymph nodes. Sections of the calves' lungs often showed as high as eight to ten megakaryocytes in a single high power field. Collections of megakaryocytes in the spleen were found in each type of animal used. The liver showed a very rare megakaryocyte and on the whole was not much involved. The greatest liver involvement occurred in the guinea pig. The bone marrow was hyperplastic and showed moderate to marked hyperplasia of the megakaryocytes. The hyperplastic marrow could not be distinguished from that of the rabbit's marrow in avian tuberculosis.

Here again lesions resembling Hodgkin's disease were not observed in animals that lived a long time with a chronic tuberculosis.

Our next study was made on guinea pigs infected with virulent human tubercle bacilli. The results were the same as in our previous experiments.

Recently we have observed the same type of lesion in lung tissue of a human being who died from an extensive acute tuberculous pneumonia, having been under sanatorium treatment for over three years. The final acute spread which caused death occurred only a few days before death. Studies of the lung sections in this case showed numerous megakaryocytes in the part of the lung involved in the recent "spread" of the tuberculous process, whereas in the more chronic lesions such cells were not observed. Here again we have the presence of megakaryocytes in the acute tuberculous process and not in the chronic.

agents being the incitant as readily as if they were present. It also enables one to understand the fact that identical tissue reactions may be observed in infectious and in non-infectious injury to the living tissues.

The finding of lesions resembling Hodgkin's disease in acute tuberculosis emphasizes the fact that it is necessary to have more than giant cells of the Sternberg type to establish a diagnosis of Hodgkin's disease. One may find in an enlarged and hyperplastic lymph node such giant cells scattered about in the tissues, and yet the lesions may not be Hodgkin's disease at all.

### SUMMARY AND CONCLUSIONS

1. Lesions resembling Hodgkin's disease are often present in acute tuberculous foci. These lesions are simply the accumulation of megakaryocytes which have emigrated from the bone marrow to the areas of damaged tissue. They are not true Hodgkin's lesions.

2. The participation of the megakaryocyte in an acute tuberculous process is emphasized. This is not specific for tuberculosis since it occurs in other pathological conditions.

3. The necessity of exercising care in the diagnosing of Hodgkin's disease from tissue sections is stressed. The presence of the Sternberg type of giant cell in tissue is not sufficient evidence to warrant a diagnosis of Hodgkin's disease.

4. Various types of virulent tubercle bacilli cause the production of the lesions which simulate Hodgkin's disease. This would seem to preclude the possibility that any one type of tubercle bacillus is the etiological agent of Hodgkin's disease.

5. Lesions suggestive of Hodgkin's disease have been observed only in acute tuberculosis. In chronic tuberculosis these lesions have not been encountered.

acute tuberculosis. These lesions we believe are simply accumulations of megakaryocytes in the tissues and are not real Hodgkin's lesions. We have not observed the pleomorphism of cells, the predominance of the Hodgkin's cell type or the other phenomena which constitute the histopathology seen in a true case of Hodgkin's disease.

We do not believe that any type of tubercle bacillus is the etiological agent in Hodgkin's disease. Neither do we believe that any infectious agent is the etiological factor. The recent report by Twort<sup>5</sup> makes such a belief more tenable than ever before. It is possible that an acute infection may in some instances upset the normal balance in such a way that Hodgkin's disease may ensue. But it is just as plausible that such an imbalance might be produced by a non-infectious agent. As long as one adheres to the dogma that a response on the part of the hematopoietic tissues, especially of the leucocytes, designates the presence of an infection within the tissues, it will remain imperative that the leukemias, Hodgkin's disease, pernicious anemia, and so on, be ascribed to the action of some pathogenic microorganism. If the above concept is logically adhered to, then all responses on the part of the blood cells signify infection. This would lead one to believe that nature has prepared for the eventual occurrence of infections by differentiating the blood cells early in embryonic life, long before there is any contact with pathogenic microorganisms. To carry this conception one step further, all inflammatory reactions must be due to the presence in the tissues of pathogenic microorganisms. Thus an abscess formed as a result of sterile turpentine injected into the tissues is the result of pathogenic microorganisms being present and is not due to the turpentine.

If, however, one believes that the function of the blood cells is to assist in the maintenance of a biological balance compatible with life, then it becomes apparent that their rôle extends far beyond the part they take in the combat against an infectious agent. Such a biological concept offers a better explanation of the acute inflammatory reaction which is seen in an animal sensitized to a sterile protein substance such as egg white, or which is produced when a sterile chemical substance such as turpentine is injected into living tissue. Thus it can be concluded that such pathological conditions as Hodgkin's disease or the leukemias may occur without infectious



## DESCRIPTION OF PLATES

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### PLATE III

FIG. 1. Hodgkin's lymph node showing two Sternberg giant cells.  $\times 1000$ .

Fig. 2. Lymph node in acute tuberculosis in a calf. Two megakaryocytes in field. Bovine tubercle bacillus infection.  $\times 1000$ .

FIG. 3. Lung in Hodgkin's disease without tuberculosis. Note two giant cells in alveolar walls.  $\times 1000$ .

FIG. 4. Megakaryocyte in alveolar wall. Acute tuberculosis in calf. Bovine tubercle bacillus infection.  $\times 1000$ .

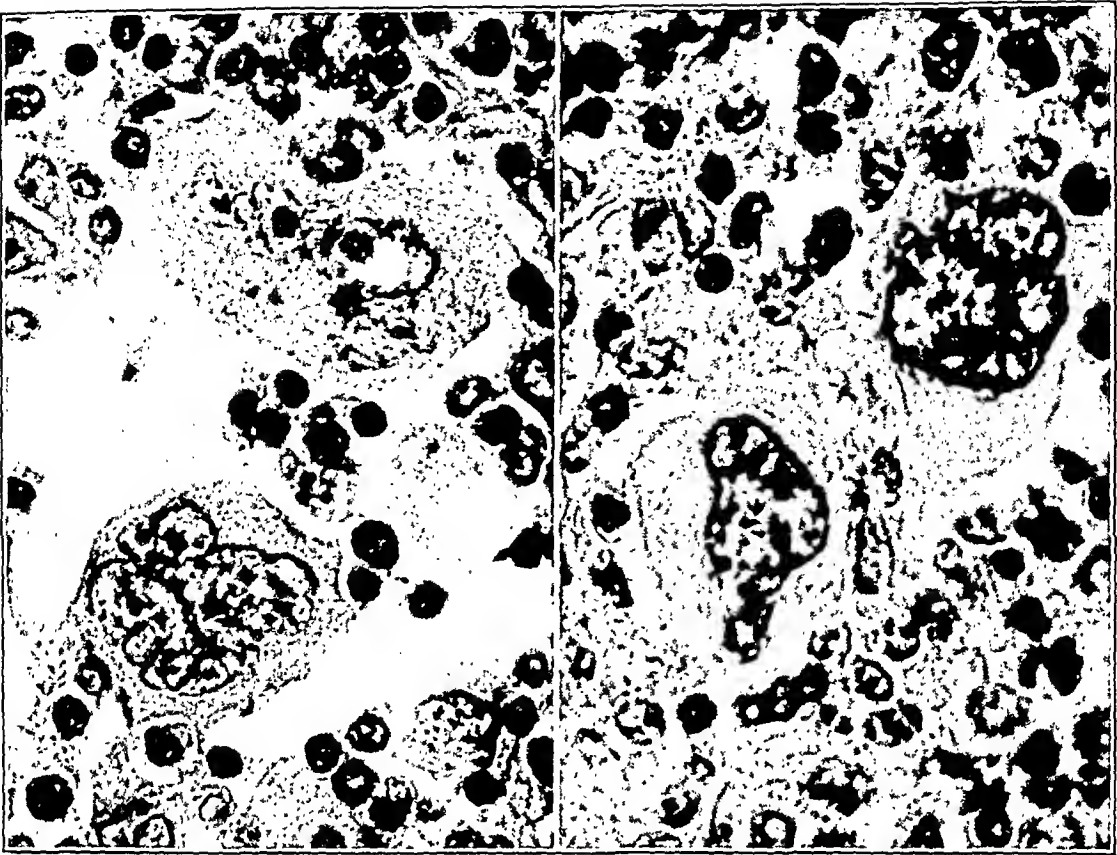
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PLATE 112

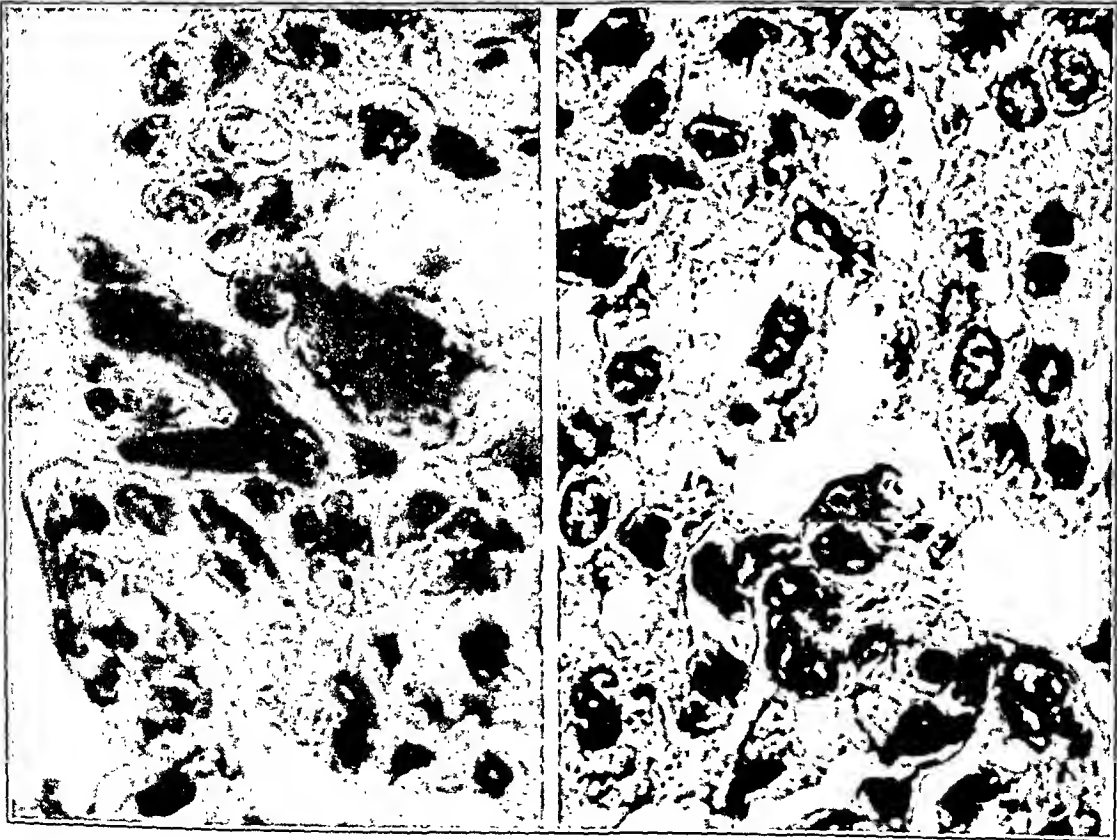
FIG. 5. Lung, calf. Acute tuberculosis. Numerous megakaryocytes in field.  $\times 1000$ .

FIG. 6. Bone marrow from rabbit. Marked hyperplasia of marrow. Note numerous megakaryocytes. Two megakaryocytes in mitosis to right of field. Bovine tubercle bacillus infection.  $\times 400$ .



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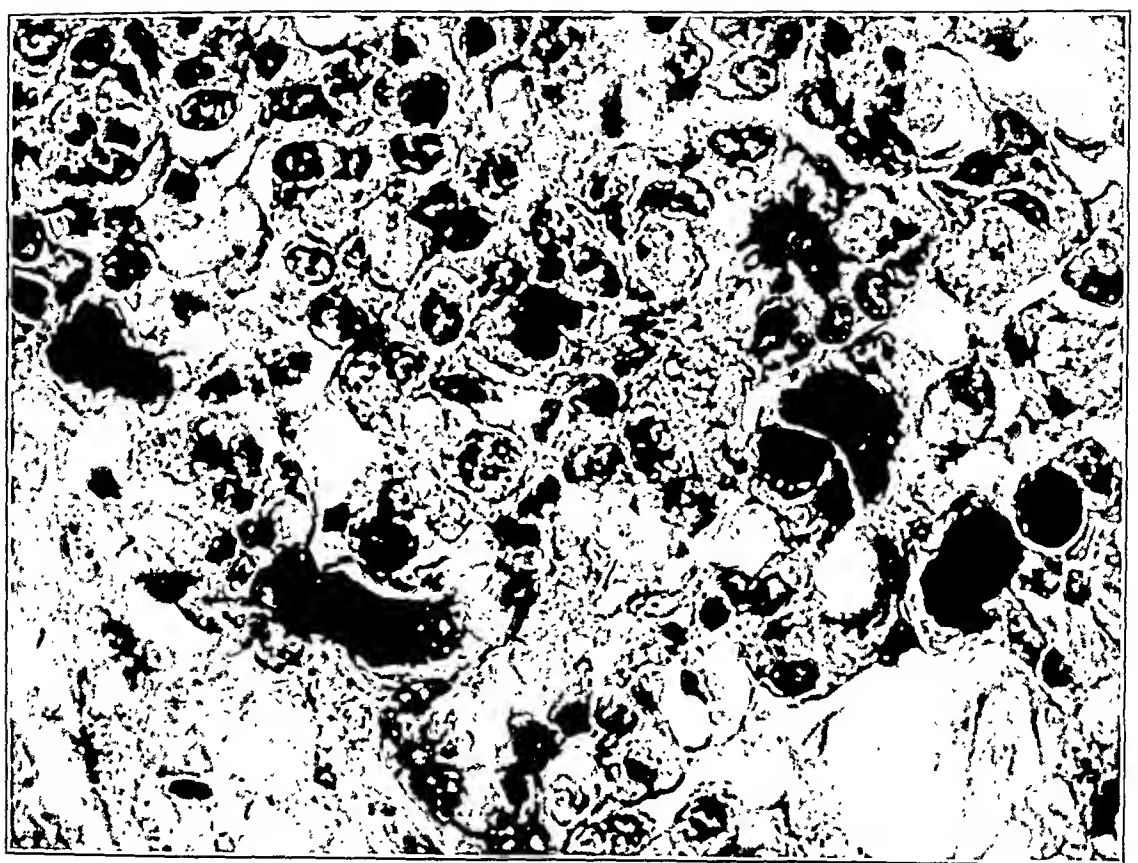
PLATE 113

FIG. 7. Human liver from case of Hodgkin's disease. Four Sternberg giant cells in center of field.  $\times 800$ .

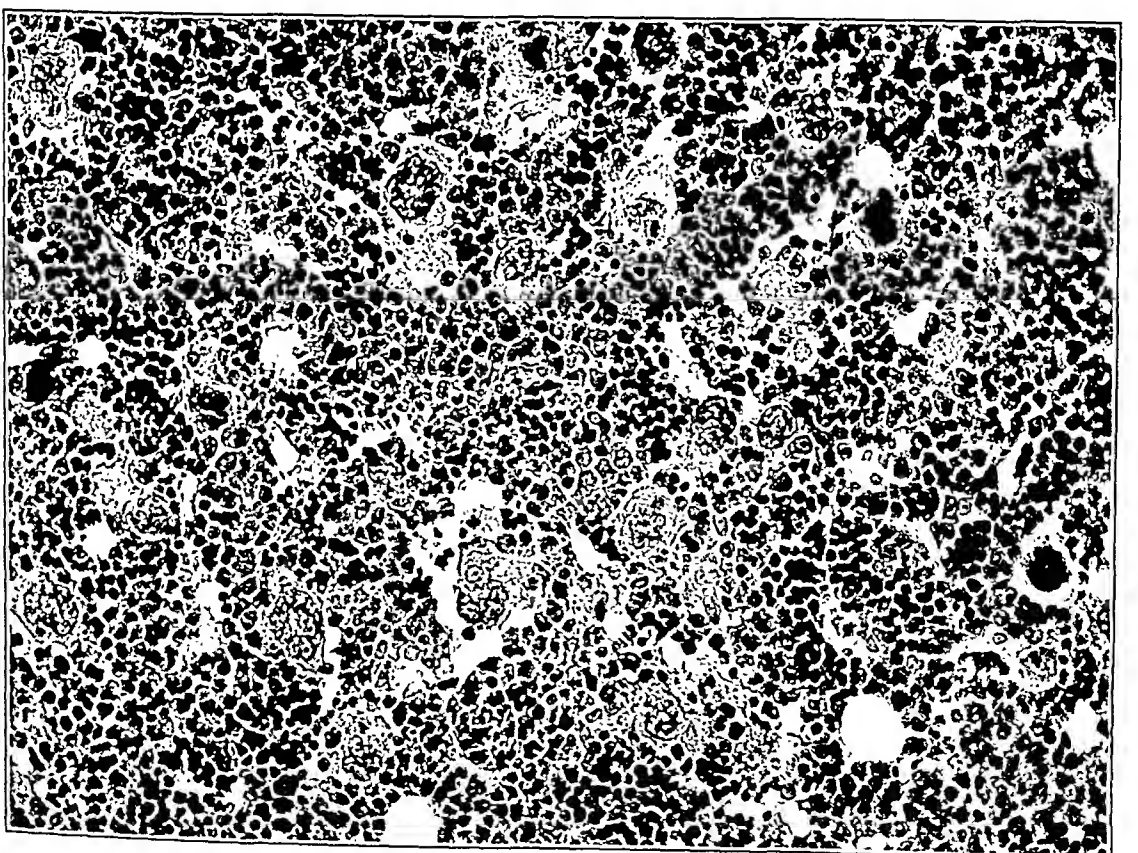
FIG. 8. Rabbit liver. Avian tubercle bacillus infection. Three megakaryocytes in field.  $\times 800$ .

FIG. 9. Human spleen. Scirrhouz Hodgkin's lesion. Two Sternberg giant cells in sinusoid.  $\times 800$ .

FIG. 10. Guinea pig spleen. Human tubercle bacillus infection. Two megakaryocytes in large sinusoid.  $\times 800$ .



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PLATE 114

FIGS. 11, 12, 13. Rabbit marrow. Megakaryocytes in process of entering circulation.  $\times 800$ .

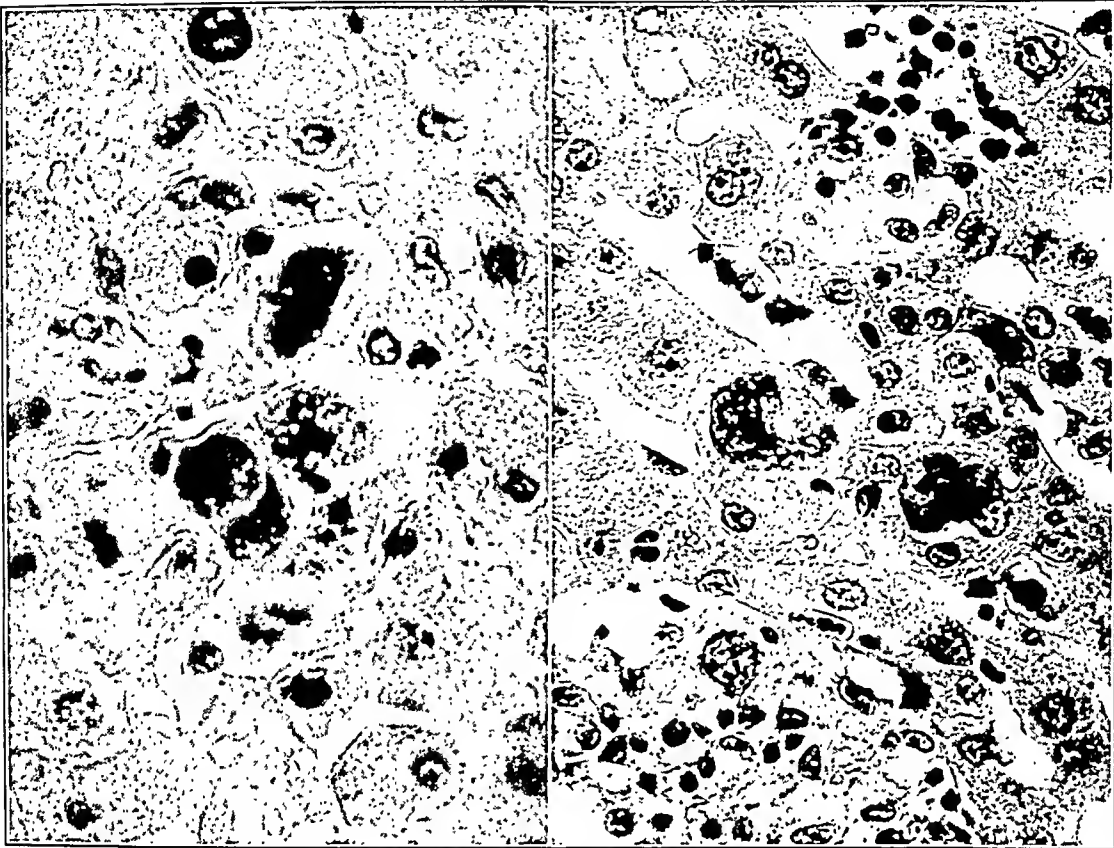
FIG. 14. Two mitotic figures in megakaryocytes in rabbit marrow. Avian tubercle bacillus infection.  $\times 800$ .

FIG. 15. Megakaryocytes in mitosis. Lung from calf. Bovine tubercle bacillus infection.  $\times 800$ .

FIG. 16. Megakaryocyte in mitosis. Spleen from rabbit. Bovine tubercle bacillus infection.  $\times 800$ .

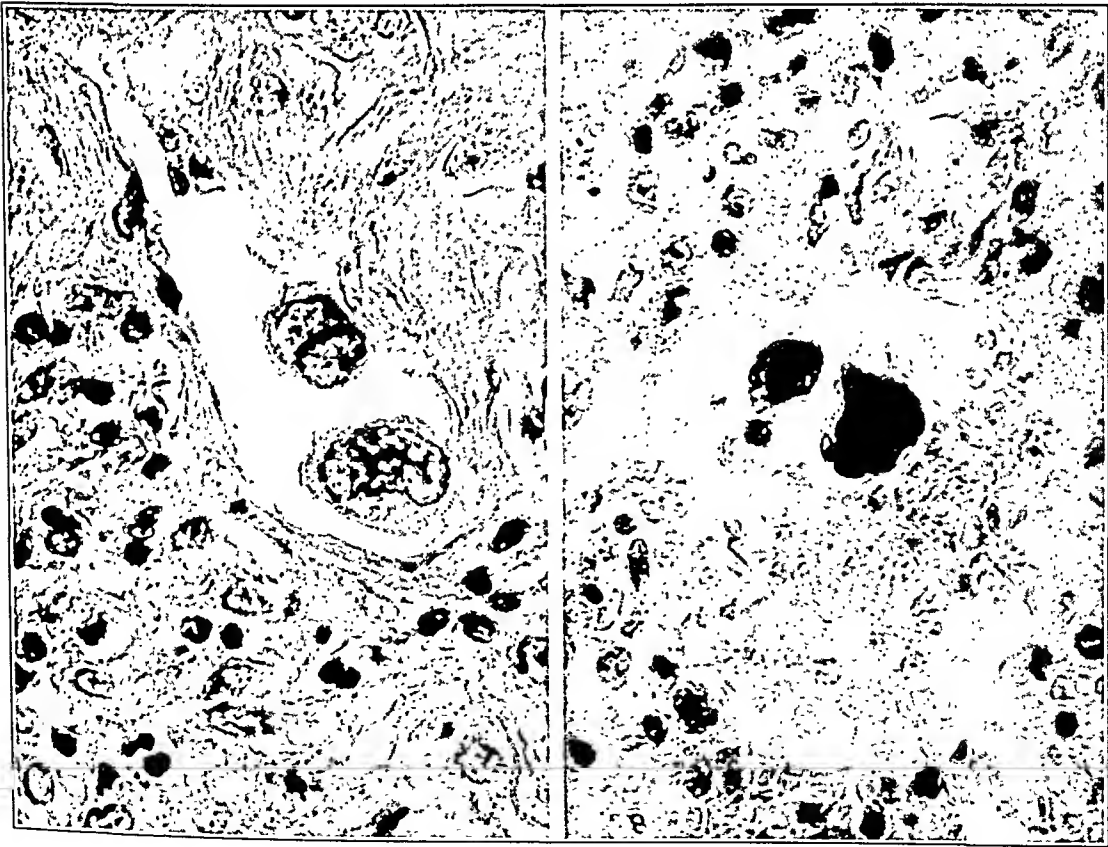
FIGS. 17 and 18. Megakaryocytes wandering about in tissue. Lung from calf. Bovine tubercle bacillus infection.  $\times 1000$ .

FIG. 19. Megakaryocytes in alveolar wall. Acute tuberculous pneumonia in human lung. Human type tubercle bacillus infection.  $\times 800$ .



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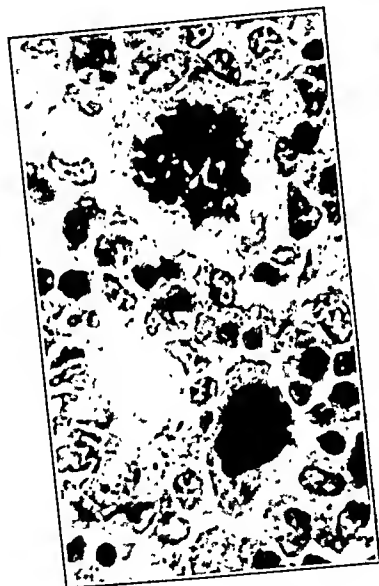
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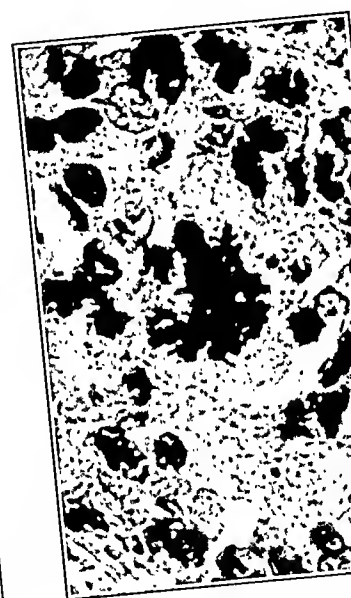
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Other investigators, Bunting and Yates,<sup>3</sup> have failed to find the tubercle bacillus frequently in Hodgkin's lesions, but have found diphtheroid organisms quite consistently. More recently, Haythorn<sup>4</sup> has obtained a culture of monilia from the tissues of a case of Hodgkin's disease.

In the study of tuberculous lesions in experimental animals we have noted the occurrence of numerous giant cells in lungs, liver, spleen and lymph nodes which we were unable to differentiate from the giant cells seen in Hodgkin's lesions. We observed these lesions suggestive of Hodgkin's disease first in rabbits inoculated with virulent avian tubercle bacilli. Upon further study we found similar lesions in guinea pigs, rabbits and calves infected with virulent bovine tubercle bacilli. We have also observed similar lesions in guinea pig and human tissues infected with virulent human type tubercle bacilli.

A thorough study of the various tissues of the experimental animals and of human tuberculous tissue led us to the conclusion that the cells which resembled the Sternberg giant cells of Hodgkin's lesions were megakaryocytes. At certain stages of acute tuberculosis there was a marked hyperplasia of the megakaryocytes in the marrow, with a wandering-out of these cells from the marrow into the circulation and thence into the various tissues involved in the tuberculous process. We have not observed this phenomenon in chronic tuberculosis, although it is possible that it exists to a slight degree.

The marked hyperplasia of the megakaryocytes in acute tuberculosis and our inability to distinguish between the megakaryocytes in the tissues and the Sternberg giant cells in Hodgkin's lesions led us to a study of the histopathology of Hodgkin's disease, with the possibility that the megakaryocyte might be the cell type primarily involved in the pathological process.

The material which we were enabled to study consisted of twenty-two autopsies and about one hundred surgical specimens.\* Unfortunately the number of bone marrow specimens which we examined were very few. Of the twenty-two autopsies, bone marrow sections were available in but six instances. In four instances femur marrow

\* We wish to express our appreciation for the kind permission of Drs. F. B. Mallory and F. Parker, Jr., to utilize the autopsy material and surgical specimens of Hodgkin's disease from the pathological laboratory of the Boston City Hospital. They granted the study of the skin nodule from a case of myelogenous leukemia also.

## AN INTERPRETATION OF THE NATURE OF HODGKIN'S DISEASE \*

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The number of contributions relative to Hodgkin's disease is so large that it is impossible to present adequately the views of the various authors in a single article, unless the paper be devoted entirely to that phase of the subject. The review of Simonds<sup>1</sup> renders another article on this topic superfluous at the present time, for little has been added in the way of further clarification of this disease since his article was published. Our references to the literature will, therefore, be confined to citing certain points of view pertinent to the discussion of Hodgkin's disease we desire to present.

It is generally conceded that there is a pathological and clinical entity which justifies the grouping of certain cases under the term "Hodgkin's disease." The features which allow such a separation are so well known that no comment is necessary.

The nature of Hodgkin's disease is still very uncertain. There are two schools extant at present. The one school maintains that the disease is neoplastic in nature. The adherents to this view maintain that the tumor is primary in the lymphoid tissues of the body.

The second school believes that Hodgkin's disease is of an infectious nature. Bacteriological studies by different investigators have led to no single microorganism as the etiological agent. The tubercle bacillus has been held to be the inciting agent by some authors. The reason for this deduction has been the obtaining of tubercle bacilli from Hodgkin's tissues. The irregularity of these findings has led to a belief that to obtain Hodgkin's lesions it is necessary to have a certain type of tubercle bacillus which has a certain degree of pathogenicity. More recently L'Esperance<sup>2</sup> has presented evidence which suggests that the avian tubercle bacillus might be the etiological factor.

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in animal marrow we naturally sought for the same cell type in the Hodgkin's marrow.

Fig. 5 shows the appearance of a considerable part of a section of vertebral marrow from a case of Hodgkin's disease without evidence of tuberculosis or of other infectious lesions. Note the considerable number of nondescript cells which resemble the cells shown in Fig. 1. Whether these are premegakaryocytes or "stem" cells, one cannot say with certainty. One may state, however, that here is present a hyperplastic marrow with undifferentiated cells predominant. No megakaryocytes are seen.

Fig. 6 gives a picture also commonly met with in this same section. Here are shown two groups of the immature cells and no megakaryocytes in the field.

Fig. 7 shows an area a short distance from Fig. 6. Note the three megakaryocytes of different size and different nuclear appearance. Note also the group of cells similar to those in Fig. 6 just above the smallest megakaryocyte.

Fig. 8 is a portion of bone marrow in the same part of the section as Fig. 6. Here are shown five megakaryocytes and no groups of cells as shown in Fig. 6.

Figs. 9, 10 and 11 are higher magnifications from the same general area as Figs. 6, 7 and 8, to show that in all probability the typical megakaryocyte is the result of fusion of several of the smaller cells. Note that in Fig. 10 the complicated nuclear structure is quite similar to that in Fig. 11. There is, however, no abundant and definite cell cytoplasm as in Fig. 11. Note also the distinct granular cytoplasm in all three figures. This is more clearly seen in Fig. 11.

Fig. 12 shows an area of fibrosis in the same section from which the above photomicrographs were taken. In this particular field there are no tumor cells. The tissue is made up entirely of fibroblasts and lymphocytes. This corresponds to the fibrotic areas commonly found in Hodgkin's lymph nodes.

Fig. 13 is from another part of the same section of vertebral marrow and shows the picture commonly seen in scirrhous Hodgkin's lymph nodes. Note the ameboid shape of the Sternberg giant cell in the center.

From the above description it will be seen that all of the complex picture of Hodgkin's disease can be demonstrated in a single section of bone marrow. The points we wish to emphasize are (1) hyper-

alone had been procured and in two instances vertebral and femur marrow had been obtained at the time of autopsy.

During this study we were privileged to examine a biopsy specimen from the skin of an individual who had the clinical and blood picture of myelogenous leukemia. This individual had numerous nodules in the skin in various parts of the body. The study of the sections from this skin nodule gave the point of departure for the interpretation of Hodgkin's disease which will be presented here.

The histopathological picture present in sections of the skin nodule was complex. In places there were groups of nondescript cells (Fig. 1). These cells were round, with a round to oval nucleus and slightly granular cytoplasm. They might be called large lymphoid cells, reticular cells or simply non-differentiated cells. They probably are the parent or "stem" cell of the other cell types noted in the section. In other areas of a single section the following cells were noted in groups: neutrophilic leukocytes (Fig. 2), eosinophilic leukocytes, megakaryocytes (Fig. 3) and nucleated red blood cells (Fig. 4). In other areas these were found comingled.

From this study we considered that the lesion was the result of the differentiation of the multipotential parent cell of the marrow into the various cell types which it produced normally. In other words there were leukopoietic, erythrocytic and megakaryogenic centers all present in the skin because of the differentiation of a common parent cell into these various cell types.

Since we were interested primarily in determining the possible relationship of Hodgkin's disease to the megakaryocyte, our chief interest centered on the bone marrow. Our findings in this tissue will be given in detail since we believe that the pathology which we observed is of real significance. In brief, it may be stated that the bone marrow in every case in which we studied this tissue was pathological. We will limit our description of the histopathology to one section of vertebral bone marrow, since all of the other sections of marrow gave the same picture to a greater or lesser degree.

In our study of the marrow reaction in experimental tuberculosis in animals we traced the megakaryocyte back to a cell somewhat larger than a myelocyte. This cell has very little cytoplasm and a relatively large, deeply staining nucleus. The cytoplasm was often very granular. Since this cell appeared to be the pre-megakaryocyte

all of these cell types have a common parent cell. Such a view is held by many authors, Bunting,<sup>5</sup> Maximow<sup>6</sup> and others. This leads one to a belief in the unitarian theory of hematopoiesis in the bone marrow. If, as we postulate, Hodgkin's disease may be a disease in which the megakaryocyte is the cell type chiefly involved, then it becomes apparent that Hodgkin's disease is closely related, genetically, to the myeloid leukemias and to the erythroblastic dyscrasias.

If one considers the derangements of hematopoiesis in the marrow from such an angle, then one recognizes that the further the process reverts to an embryonic state the greater will be the difficulty and the uncertainty of identification of the cells seen either in sections of tissue or in blood smears. It would seem probable that the parent cell of the megakaryocyte is a cell, which in its most immature state could not be successfully differentiated from a similar cell, which may be the parent cell of the erythrocyte or of the neutrophile. In other words, the more immature the cells of the marrow, the greater the uncertainty as to what type of cell they may become in their maturation. It is commonly known that in acute leukemic conditions it is, at times, extremely difficult, if not impossible, to determine with certainty whether the disease is of the myeloid or the lymphoid type. Some cases of Hodgkin's disease have been known to develop "lymphoid" leukemia. Since, to us, it appears that Hodgkin's disease is closely related genetically to the leukemias, it is not inconsistent that the leukemic manifestation should be an integral part of the disease process. From our study it would seem plausible that in this leukemic manifestation the cells may be very immature and in such a state they might be easily confused with immature parent cells of the lymphoid tissues. Until such time as the "stem" cell of the lymphoid tissue can be unqualifiedly distinguished from the "stem" cell of the marrow, it would seem that one is not justified in being dogmatic on the subject. In such cases of Hodgkin's disease as develop a leukemic blood pressure, we suggest a careful study of the cell types to establish, if possible, the origin of the lymphoid-like cells, and not remain content with saying they are of lymphoid origin simply because we know that the lymph nodes are very commonly involved in the disease.

The report of Minot<sup>7</sup> is of interest relative to the presence of megakaryocytes in the circulation in cases of myelogenous leukemia. His observation of immature megakaryocytes and of abnormal plate-

plasia of the marrow with a marked increase of immature cells which probably are the progenitors of megakaryocytes; and (2) presumptive evidence that the giant cells (megakaryocytes) are the end-result of fusion of several premegakaryocytes.

Mitotic figures were observed quite often in the hyperplastic marrow. The majority of mitotic figures were in the small parent cell. Occasionally one found a complex multiple mitotic figure in the large megakaryocyte. These observations also hold for the lesions found in other tissues in the body.

Since the Hodgkin's lesions, as seen in the various tissues of the body, differ in no essential respect from those described above in the bone marrow, further description would seem unnecessary. There is one point relative to the tumor cells that should be emphasized, and that is their ability to wander about in the tissues. This was shown to a striking degree in some of the surgical specimens which were promptly placed in Zenker's solution or in 10 per cent formalin.

## DISCUSSION

Any deliberation on diseases in which the blood-forming tissues of the body are chiefly concerned must necessarily be many-sided because of the complexity and the distribution of the tissue under consideration. We have observed but little in the pathology of Hodgkin's disease that has not been recorded in the literature already. The interpretation of the disease which we wish to present departs in certain respects from that presented in the literature. Our interpretation is given because to us it clarifies to some degree the nature of the disease. We are well aware of the fact that this interpretation departs rather radically from precedent and from authority. However, from the data we have at hand, our deductions appear logical to us. We do not believe that our views are final and we have no intention of being dogmatic in our statements.

The histopathology found in a nodule from the skin of an individual who had the clinical and hematological syndrome of myelogenous leukemia has a very important bearing on the interpretation of Hodgkin's disease given in this article. The ability to demonstrate in a single section of this nodule the various cell types, in groups as well as comingled, which are known to be produced in the bone marrow is, we believe, of great significance. It suggests that



Symmers<sup>10</sup> regards Hodgkin's disease as a systemic disease in which all of the hematopoietic tissues of the body are involved. He, as well as others, has noted the marked abnormality of the bone marrow. According to this author the disease is neither infectious nor neoplastic in nature. If one establishes too rigid a formula to which all malignancies must conform, then it would seem that no dyscrasia of the blood could be considered as a malignancy. There is no tissue in the body which is so pleomorphic or so mobile as the hematopoietic tissue. Therefore, in considering this tissue it would seem inconsistent to regard it in the same way as one would the epidermis. In a malignancy of the epidermis the essential considerations are (1) the autonomy of the newgrowth, and (2) the surety of the production of epidermal cells wherever the autonomatous cells may metastasize. These criteria are present in the leukemias, erythroblastic dyscrasias and Hodgkin's disease, if one considers primarily the characteristics of the parental cell of the bone marrow. The varied cellularity of these diseases would seem to be due to the fact that the parental cell is multipotential instead of unipotential as in the case of an epithelioma.

With the above discussion in mind one may logically construct the following schematic outline of hematopoiesis.

This schematic representation includes all that is, at present, accepted facts relative to hematopoiesis and ideas which, at present, are plausible although not proved. While there are controversial points in the concept, still it gives a workable, logical formula which enables one to understand more lucidly the various manifestations one observes in abnormal hematopoietic tissues and circulating blood. A recognized controversial point, to mention but one, is the placing of the monocyte as arising from the lymphoblast. Some authors hold that this cell arises from fixed connective tissue phagocytes, while others maintain that it is of bone marrow origin. Certain observations of the author (unpublished) lead him to believe that the lymphoid tissue (including the spleen) is the seat of origin of the monocyte. In an unsolved question such as this, one must admit the possibility that any point of view may be correct until unquestionable proof is established of the exact origin of the cell. Too much reliance must not be placed, however, upon a positive oxidase reaction or a certain arrangement of supravital staining granules, for the parental cell may not show these characteristics.

lets tends further to establish the unitarian theory of hematopoiesis in the bone marrow. The finding of nucleated red blood cells may be explained on the same basis. The presence of nucleated red blood cells and of myelocytes in the blood stream in Hodgkin's disease, and of abnormal platelets and of myelocytes in cases of grave erythroblastic derangements, all point toward the probability that there is a single parent cell for hematopoiesis in the marrow. This cell must be multipotential. The great variations which are manifested in dyscrasias of the bone marrow should logically lead to a study of the factors which determine the development of the marrow cells mainly along the line of neutrophiles in one case and along the line of erythrocytes in another, rather than to a superficial and artificial separation of these manifestations into distinct pathological or clinical entities.

While a classification of the pleomorphic derangements of hematopoiesis is necessary, such a classification, as far as the bone marrow is considered, should be used merely to indicate the predominant phase of a complex pathological process in which, fundamentally, a single type, multipotential cell is concerned.

Recent reports by Furth and Stubbs <sup>8, 9</sup> on leukosis in fowl bear directly on our discussion. They state that they have found two distinct types of disease, myelogenous and erythroblastic. A very significant feature of their findings is that when blood from the myelogenous type is injected into normal fowl, the disease reproduced is not uniform in type. Some of the fowl develop the myelogenous type, others show the erythroblastic type, while in still other fowl injected with blood from the same source a mixed type of disease becomes manifest. The same lack of uniform results occurs when blood from the erythroblastic type is injected into normal fowl. These observations point very strongly to a common multipotential parent cell for the complex disease process which they have found in the leukosis of fowl. It is of interest, also, that they have not noted the occurrence of typical lymphocytic leukemia in their studies. This would suggest that, in the fowl at least, there is a distinct difference between the leukemias of bone marrow origin and those of lymphoid tissue origin. There is a certain amount of evidence to suggest that there is also a distinct difference between these two types in the human being. That the most embryonic types of these diseases may closely resemble each other is probable.

It will be noted that in the schematic outline under consideration a line has been drawn from the "stem" cell of the marrow to the "stem" cell of the lymphoid tissue with arrows pointing in either direction. This line is to suggest that there may be a possibility of the myeloblast assuming the function of the lymphoblast, or *vice versa*. Whether this does actually occur in mature life would appear impossible to prove since, at present, no sure differentiation can be made between a "stem" cell of the lymphoid tissue and a similar cell of the bone marrow. If it is possible for these cells to assume functions foreign to their usual rôle, then one must admit the possibility of the abnormal cells in Hodgkin's lymph nodes being derived from lymphoblasts. However, such an occurrence appears remote to the author for one does not see erythrogenic or leukopoietic centers commonly in these lesions. It would seem illogical for the lymphoblast to take over but a small part of the function of the myeloblast.

In considering such an outline as given above one can conceive that the bone marrow response in a case of severe infection might become so abnormal that a neoplastic condition would be simulated. That such conditions do arise should not lead one to hypothecate that all blood dyscrasias are the result of infection and that there is no true non-infectious neoplasia of the hematopoietic tissues. Such reasoning applied to other tissues we know would lead to erroneous conclusions. For instance, over-production of connective tissue in tissue repair we know seldom ends in a true neoplasm, although the gross and microscopic appearance might closely simulate an early neoplasm. To conclude that neoplasms never arise during the course of tissue repair we know would be without foundation. Autonomy of cell growth is a fundamental essential for neoplastic development. To recognize such autonomy at its inception is, at present, impossible. This is just as true for the hematopoietic tissue as it is for the fixed connective tissue. Different degrees of malignancy are recognized in neoplasms. This is in all probability, as true for the hematopoietic tissues as for any other tissue. The majority of medical men admit that neoplasms of the skin, bone and other tissues arise without infections playing any rôle. We can see no logical reason why the same may not be true of the hematopoietic tissue.

Those who are of the opinion that Hodgkin's disease is infectious in nature also regard all of the leukemias as being caused by some

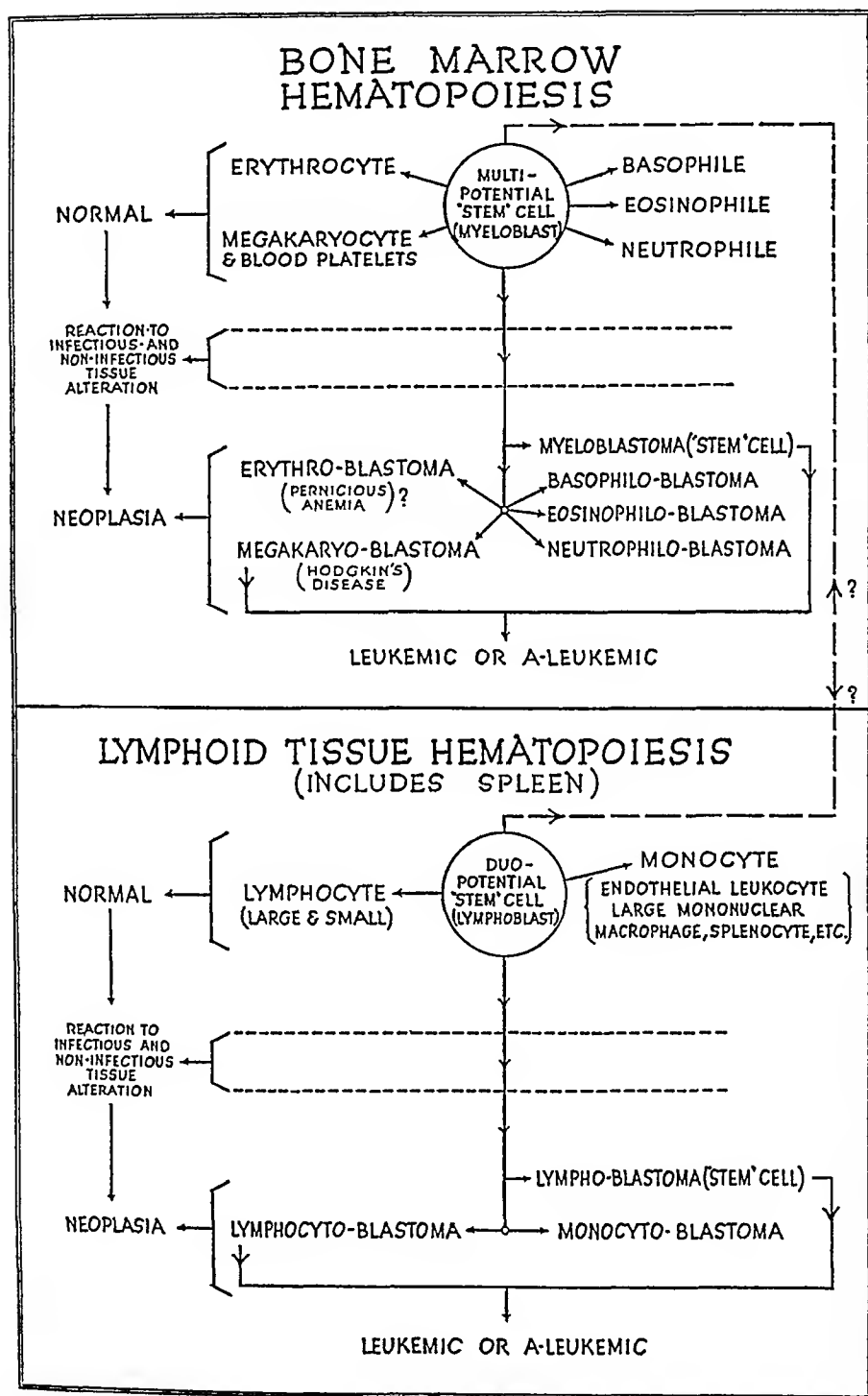


Chart 1

in the circulating blood in Hodgkin's disease. Since Wright <sup>12</sup> has so clearly demonstrated that megakaryocytes are the cells from which platelets arise, the findings relative to the platelets in Hodgkin's disease fit in readily with the disease being primarily an involvement of the megakaryocyte.

The finding of increased or abnormal platelets is not peculiar to Hodgkin's disease. Such a phenomenon is also noted in acute lobar pneumonias, in acute tuberculosis and other acute processes. It would appear that blood platelets play a far more important rôle in the response to acute damage to tissues than is generally appreciated.

The finding of neutrophilic or eosinophilic infiltration in Hodgkin's lesions need not necessarily indicate the presence of an infectious agent. Their presence can just as logically be explained by the necrosis of the tumor cells.

Another common occurrence in Hodgkin's lesions is fibrosis. This occurs in bone marrow, as well as in lymph nodes and other tissues. From a purely theoretical consideration one may explain this fibrosis in the following way. If the tumor cell type is the megakaryocyte, then there will be a marked production of blood platelets in the tumor nodules. Blood platelets are closely connected with the formation of fibrin. Wherever there is much fibrin formation there is an organization of the fibrin by fibroblastic proliferation. This would lead to the marked fibrosis commonly seen in the more chronic Hodgkin's lesions.

By placing the primary lesion of Hodgkin's disease in the bone marrow one can more easily understand the irregular distribution of the lesions in the body. Lesions outside of the marrow may be considered metastatic tumor growths. These metastases, in all probability, take place through the blood stream. From a study of the circulating blood which we are pursuing at the present time, we have a certain amount of evidence that this is true. This study will be reported at a later date. Minot <sup>7</sup> has also noted immature megakaryocytes in the blood in Hodgkin's disease. Regarding the lymph node involvement as a metastasis through the circulation, one can understand the independent and spontaneous enlargement of lymph nodes, simultaneously or at different dates, in different parts of the body such as the groin and the axilla. Why the tumor finds lymphoid tissue especially suitable for metastatic growth remains at present an unsolved question. The same is true of the reason why

pathogenic microörganism. We believe that such an interpretation of these dyscrasias is due to the fact that leukocytic responses of all types have come to be regarded almost wholly as indicative of an infectious process. The significance of the response on the part of the blood cells as representing a broad biological response, common to both infectious and non-infectious tissue damage, seems to have received but little acclaim. It seems that we are more easily impressed by the activities of the pathogens than by the activities of the body tissues in their attempt to adjust themselves to a wide range of changing environments. At present the neutrophile is considered pathognomonic of an acute infection by the large majority of medical men. Since the parent cell of the neutrophile appears to be the same as the parent cell of the erythrocyte, it would appear unreasonable to pick out the neutrophile any more than the erythrocyte as pathognomonic of acute infection. Yet it is doubtful if anyone would look upon the presence of immature erythrocytes in the circulation as pathognomonic of acute infection. It would seem more logical to look upon all of the cells of the hematopoietic tissue as carrying on a definite physiological or metabolic function in the body, irrespective of the presence or absence of any infectious agent. One might say that the rôle of each leukocytic type is primarily to care for damage to the body tissues of one type or another, irrespective of whether this alteration is produced by a tubercle bacillus, a pneumococcus, necrotic tumor tissues, croton oil or sterile normal salt solution. Undoubtedly the determination of the definite function of each leukocytic type far antedates the presence of any pathogenic bacterium in the tissues. Logic suggests an interpretation of the response of the marrow and lymphoid cells upon the basis of the type of alteration in the body to which they respond, rather than upon the presence or absence of infection. One grants that infectious agents damage tissue, but so will boiling water. In either instance the leukocytic response will tell with considerable accuracy the degree and type of damage the tissues have sustained. If one is willing to regard the response on the part of the hematopoietic tissue in such a light, then it becomes evident that the leukemias of one type or another may properly be removed from the infectious diseases and placed logically under neoplasms.

Many observers, Bunting,<sup>11</sup> and others, have noted the marked increase of blood platelets and the presence of enormous blood platelets

sumptive evidence is found which suggests that the small pre-megakaryocytes fuse to form the typical megakaryocyte. Such a finding allows an understanding of the difficulty in demonstrating various stages between the premegakaryocyte and the typical adult cell. It would seem logical to consider the premegakaryocyte or the immature megakaryocyte as analogous to the thrombocyte in the blood of birds and reptiles. Minot regards megakaryocytes with small nuclei as degenerative forms. From our observations the presence of several small nuclei in a megakaryocyte is not a criterion of degeneration of the cell.

From the above discussion it would seem as though Hodgkin's disease should be removed from the group of infectious diseases and placed as a malignancy of the hematopoietic tissues of the bone marrow. This also would imply the removal of this disease as a primary neoplasm of the lymphoid tissue. The involvement of tissues outside of the bone marrow could be logically regarded as metastatic growths.

A certain amount of evidence is presented which suggests that the cell primarily involved in the pathology of Hodgkin's disease is the megakaryocyte. To denote that the predominant phase of Hodgkin's disease, which distinguishes it from the other bone marrow dyscrasias, is the primary involvement of the megakaryocyte, the term "megakaryoblastoma" is suggested to designate real Hodgkin's disease.

#### SUMMARY AND CONCLUSIONS

1. Evidence is presented which suggests that Hodgkin's disease is a malignancy of the bone marrow. The type cell appears to be the megakaryocyte.

2. The developmental cycle of the megakaryocyte is presented. It would seem that the typical megakaryocyte is the result of fusion of several premegakaryocytes.

3. The histopathology of Hodgkin's disease is a pleomorphic aggregation of cells which represent the developmental cycle of the megakaryocyte. It is not essential to have fibrosis or eosinophilic or neutrophilic infiltration to establish the diagnosis of Hodgkin's disease.

4. The involvement of lymph nodes and other tissue outside of the bone marrow appears to be metastatic tumor growth.

some lymph nodes or other tissues, such as the liver, are involved more frequently than others.

A study of the lymph node lesions gives evidence that the consecutive enlargement of lymph nodes in a certain area, such as the cervical nodes, is due to metastasis from one lymph node to its neighbor. One can quite frequently find numerous groups of the tumor cells within lymph ducts.

In lymph nodes, especially in early growth, one finds the tumor cells scattered in different parts of the organ with the architectural structure of the node fairly normal. Older lesions will show practically the whole of the node occupied by the tumor growth. If it is appreciated that the megakaryocyte can wander about in the tissues, then it is easy to understand the diffusion of the tumor throughout the node in the early stages.

Since megakaryocytes are commonly found in tissues in diseases other than Hodgkin's disease, diagnosis cannot be made by simply finding giant cells of the Sternberg type. Rather it is essential to have a pleomorphism of cells which represent the developmental cycle of the megakaryocyte. This pleomorphism ranges from the small parent cell to the typical megakaryocyte.

One finds in hyperplastic marrow in disease other than Hodgkin's the pleomorphism of cells representative of the developmental cycle of the megakaryocyte. This phenomenon, however, is much more prominent in the bone marrow of Hodgkin's disease than in other hyperplastic marrows. We have not found in tissues outside of the bone marrow the pleomorphism of cells representative of the developmental cycle of the megakaryocyte in diseases other than Hodgkin's disease or in certain cases of "stem" cell leukemia of the bone marrow. While neutrophilic and eosinophilic infiltration and fibrosis are commonly met with in Hodgkin's lesions, they need not be present. Their presence is not necessary to establish the diagnosis of Hodgkin's disease from a section of tissue.

The study of the developmental cycle of the megakaryocyte in the marrow in acute tuberculosis has led us to appreciate that megakaryocytes do not necessarily arise as such. One finds the small immature cell, which apparently is the forerunner of the typical megakaryocyte, and the next thing one observes readily in the hyperplastic marrow is the typical megakaryocyte. From a study of the marrow in Hodgkin's disease a certain amount of pre-



## DESCRIPTION OF PLATES

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### PLATE 115

FIG. 1. Area of undifferentiated cells in a skin nodule from a case of myelogenous leukemia.  $\times 800$ .

FIG. 2. Area of neutrophils in same lesion as Fig. 1. Note a few eosinophiles and a megakaryocyte in lower part of field.  $\times 800$ .

5. Evidence is presented which tends to prove that all blood cells arising from the marrow have a common parent cell.

6. The term "megakaryoblastoma" is suggested to designate true Hodgkin's disease.

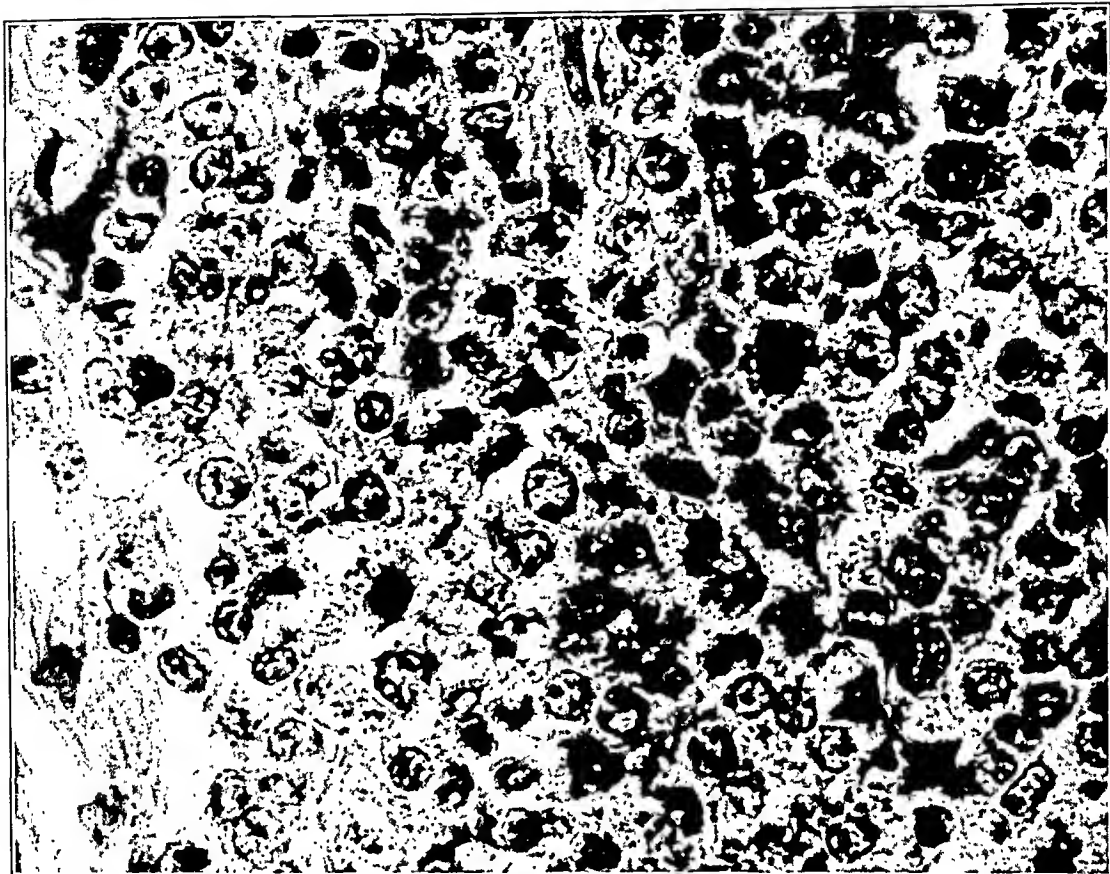
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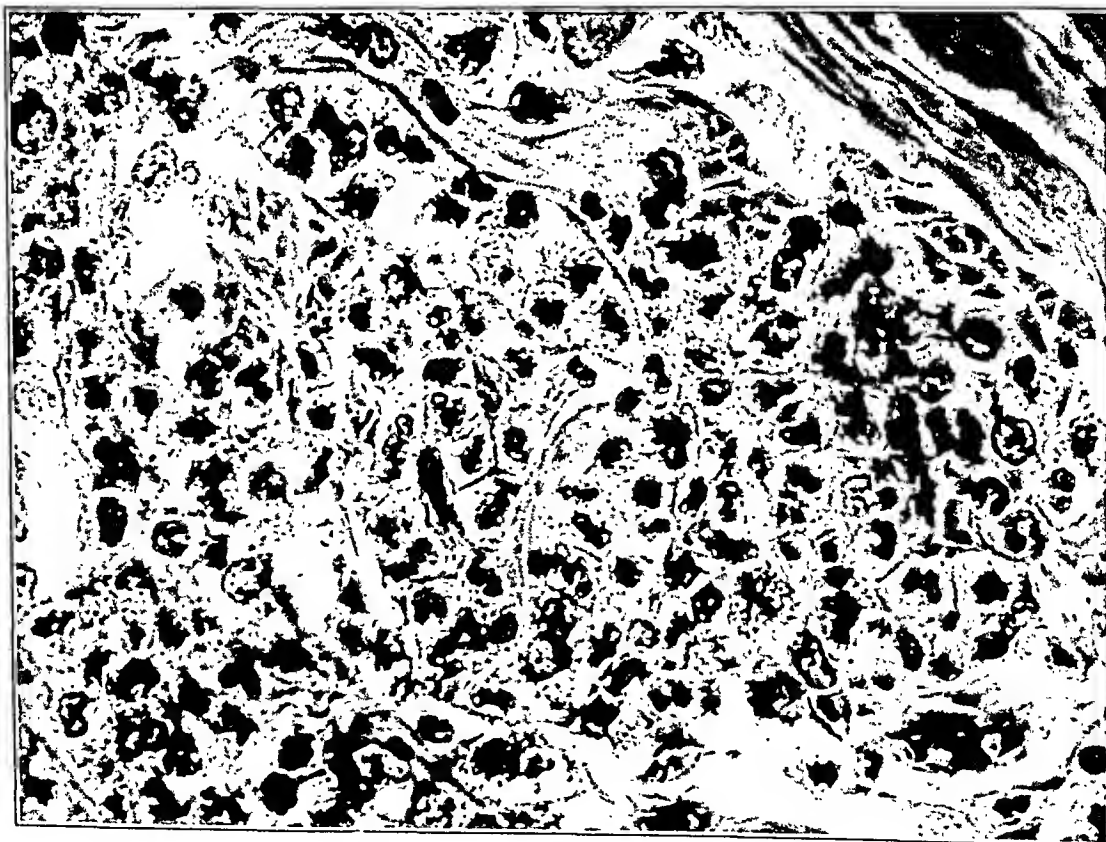
PLATE 116

FIG. 3. Megakaryocytes in same lesion as Fig. 1. Note eosinophile just below second megakaryocyte from right. Note also the numerous projections at the periphery of the megakaryocytes.  $\times 800$ .

FIG. 4. Erythrogenic center in same lesion as Fig. 1. Note the nucleated red blood cells and the undifferentiated parent cells.  $\times 800$ .



I



2

Medlar

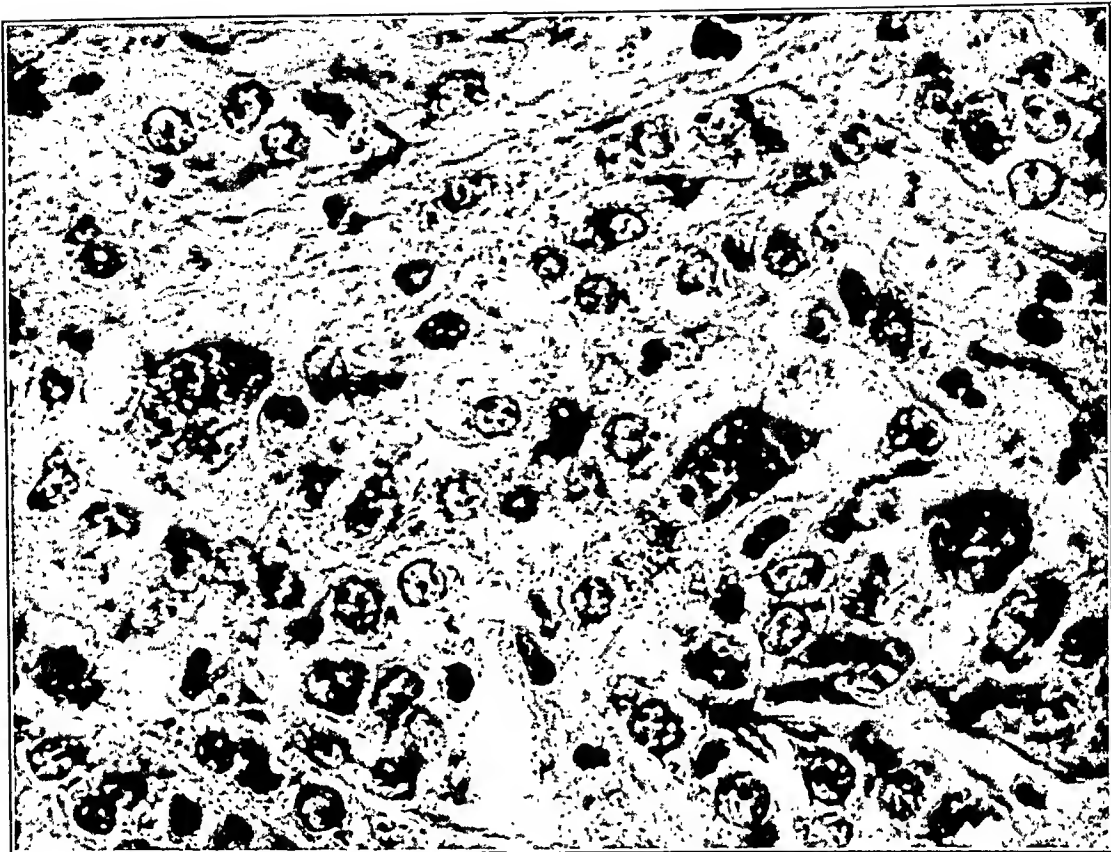
Nature of Hodgkin's Disease

PLATE 117

FIGS. 5 to 13 are all from a single section of vertebral marrow from a case of Hodgkin's disease.

FIG. 5. Hyperplastic marrow showing undifferentiated cells, quite similar to those shown in Fig. 1, scattered throughout the field. No megakaryocytes present.  $\times 800$ .

FIG. 6. Note two distinct, and one indistinct clump of undifferentiated cells in central portion of field. No megakaryocytes.  $\times 800$ .



3



4

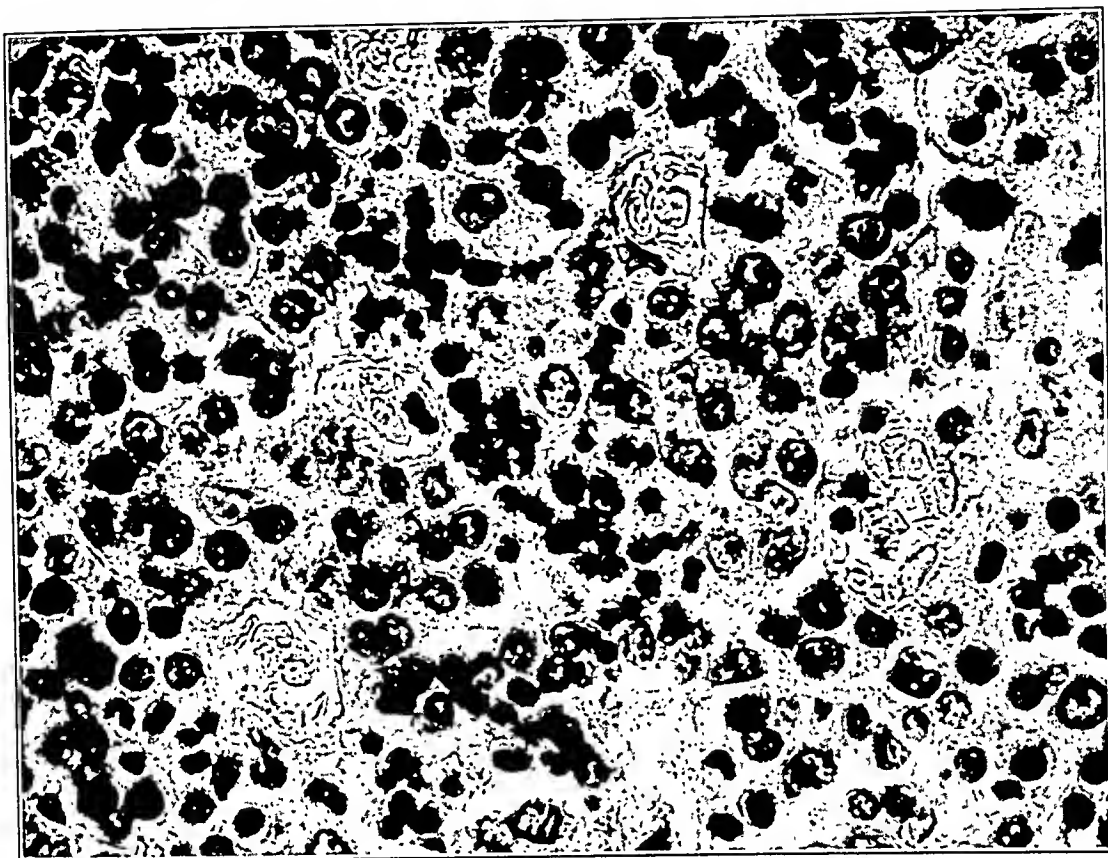
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Nature of Hodgkin's Disease

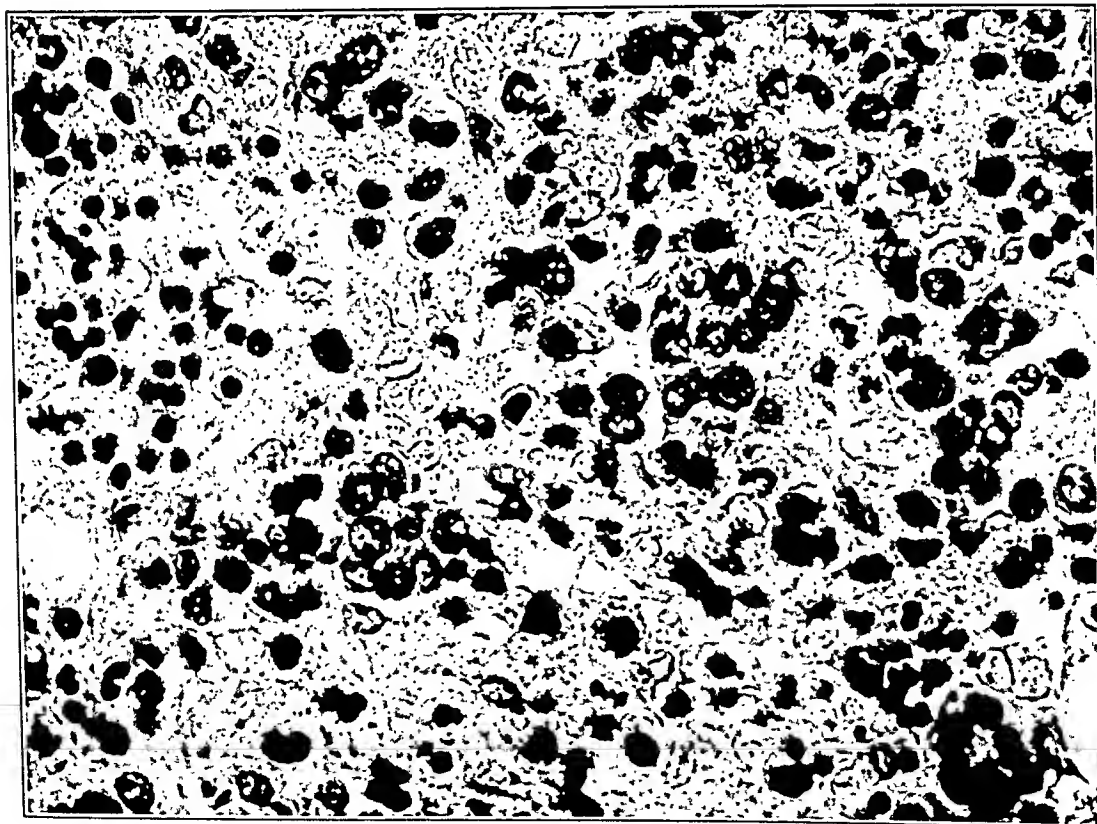
PLATE 118

FIG. 7. Three megakaryocytes and three clumps of cells in field.  $\times 800$ .

FIG. 8. Six megakaryocytes in field. No clumps of cells.  $\times 800$ .



5



6

Medlar

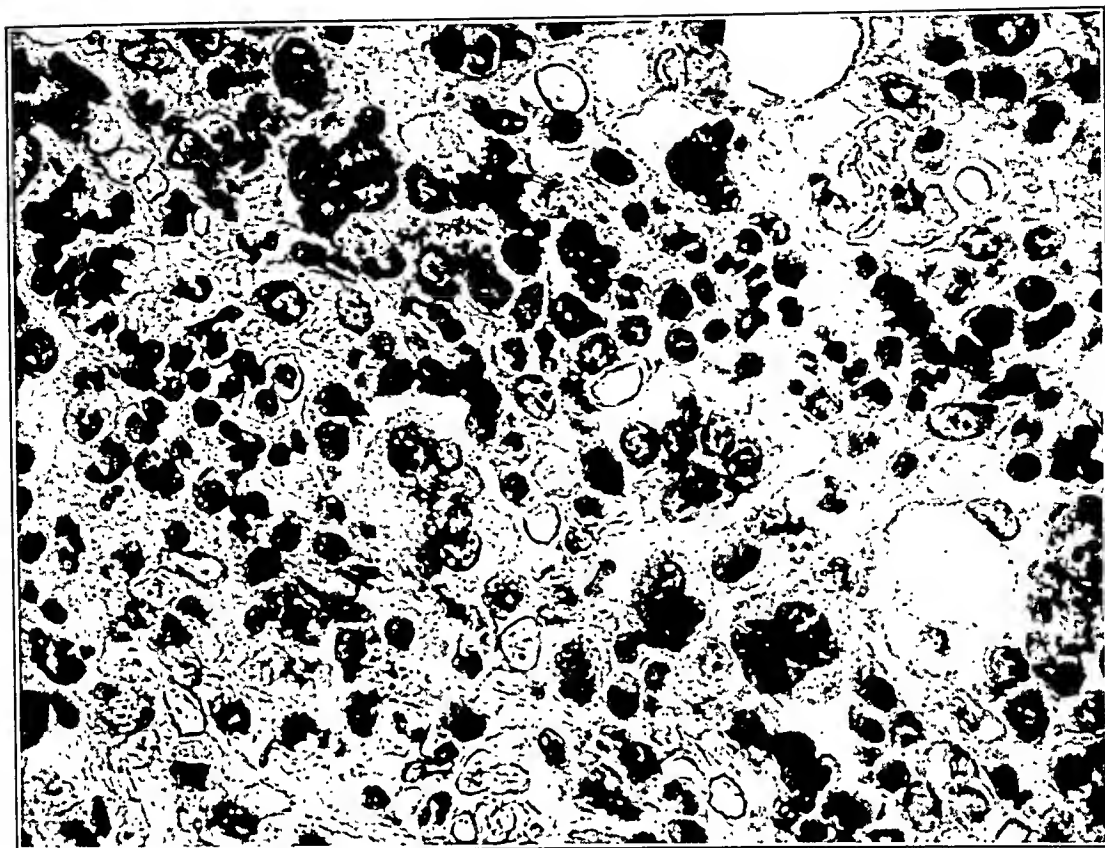
Nature of Hodgkin's Disease



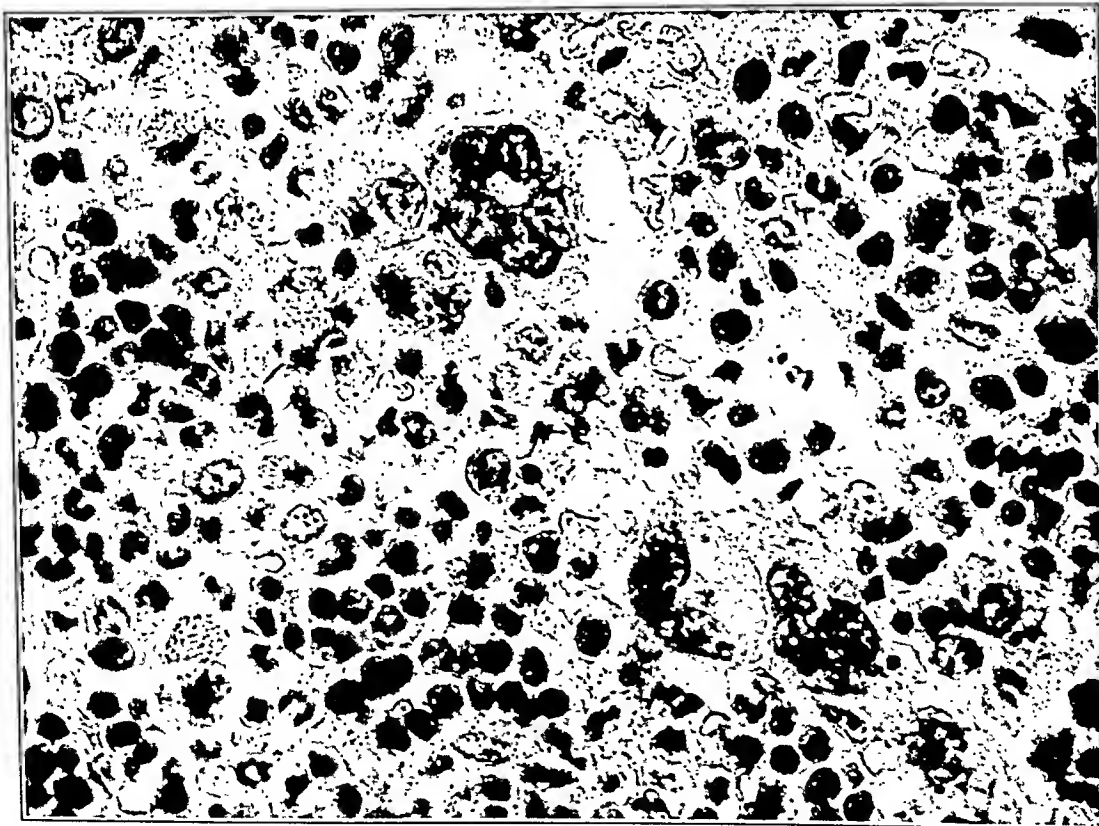
PLATE 119

FIG. 9. Clump of cells in Fig. 6. Note granular cytoplasm and separate nuclei.  
× 1500.

FIG. 10. Note apparent fusion of nuclei giving nuclear structure quite similar to that in Fig. 11. Note granular cytoplasm. Note also that cytoplasm is much less than in Fig. 11. × 1500.



7



8

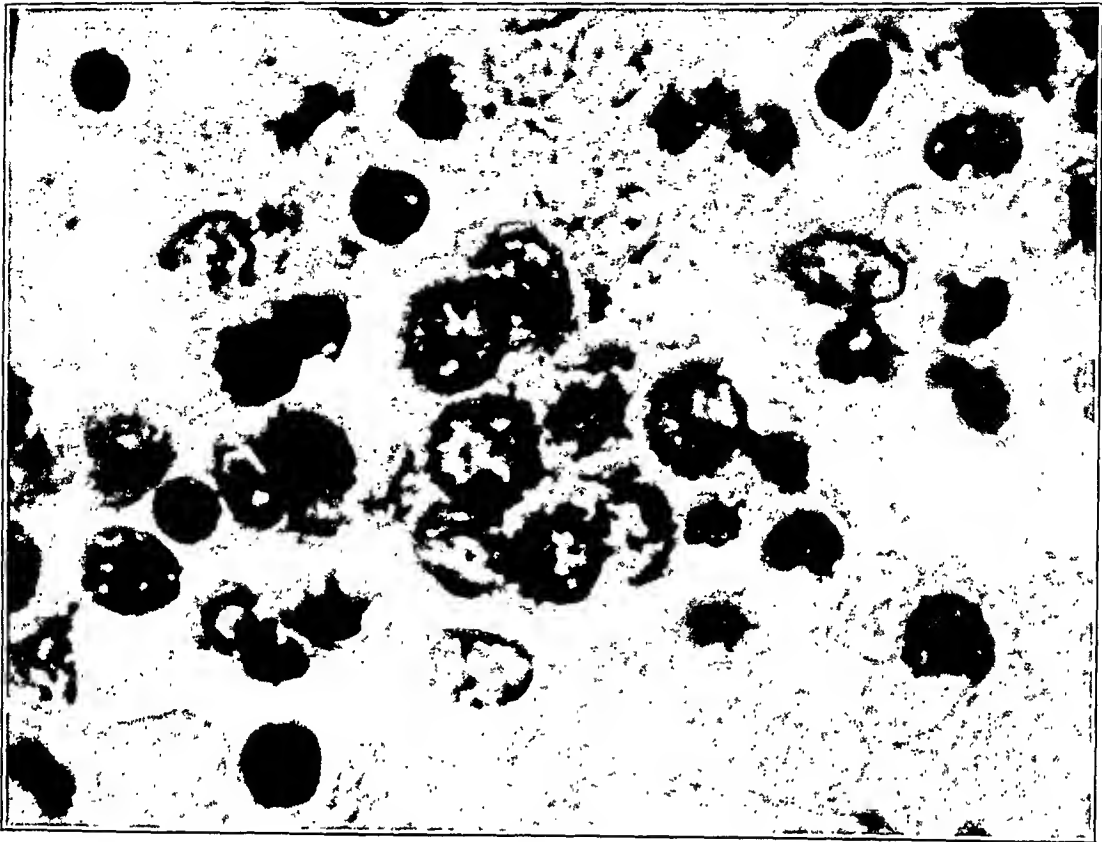
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Nature of Hodgkin's Disease

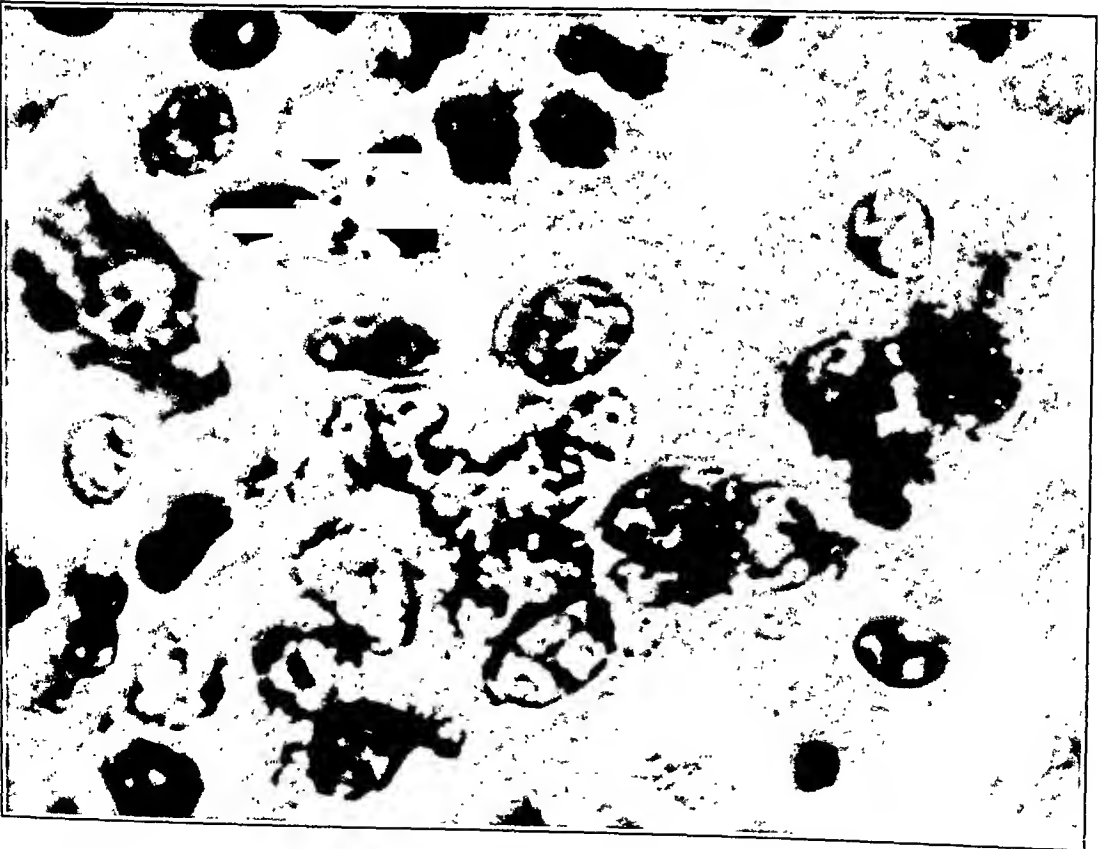
PLATE 120

FIG. 11. Megakaryocyte; nuclei are not all fused. Note abundant and granular cytoplasm.  $\times 1500$ .

FIG. 12. Area of fibrosis; tissue composed entirely of fibroblasts and lymphocytes.  $\times 500$ .



9



10

Medlar

Nature of Hodgkin's Disease

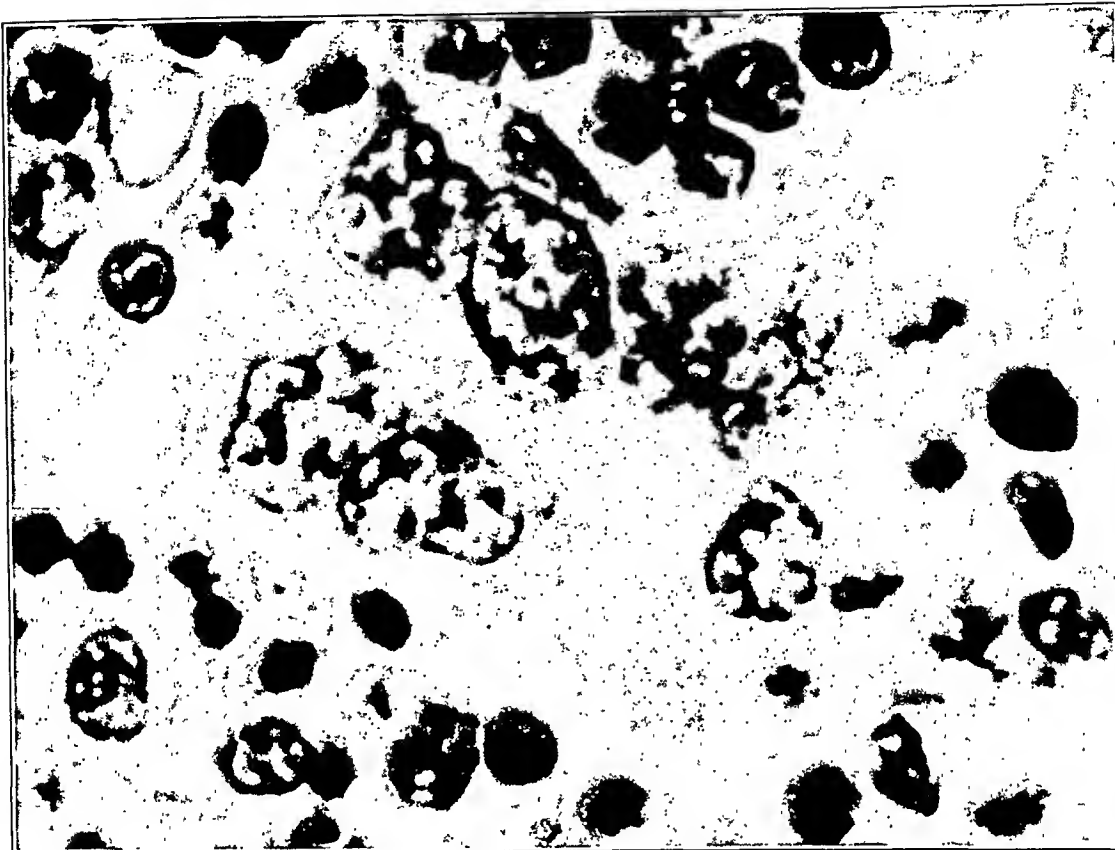
PLATE 121

FIG. 13. Typical scirrhou Hodgkin's lesion from same section as above photomicrographs. Note ameboid shape of giant cell in field.  $\times 800$ .

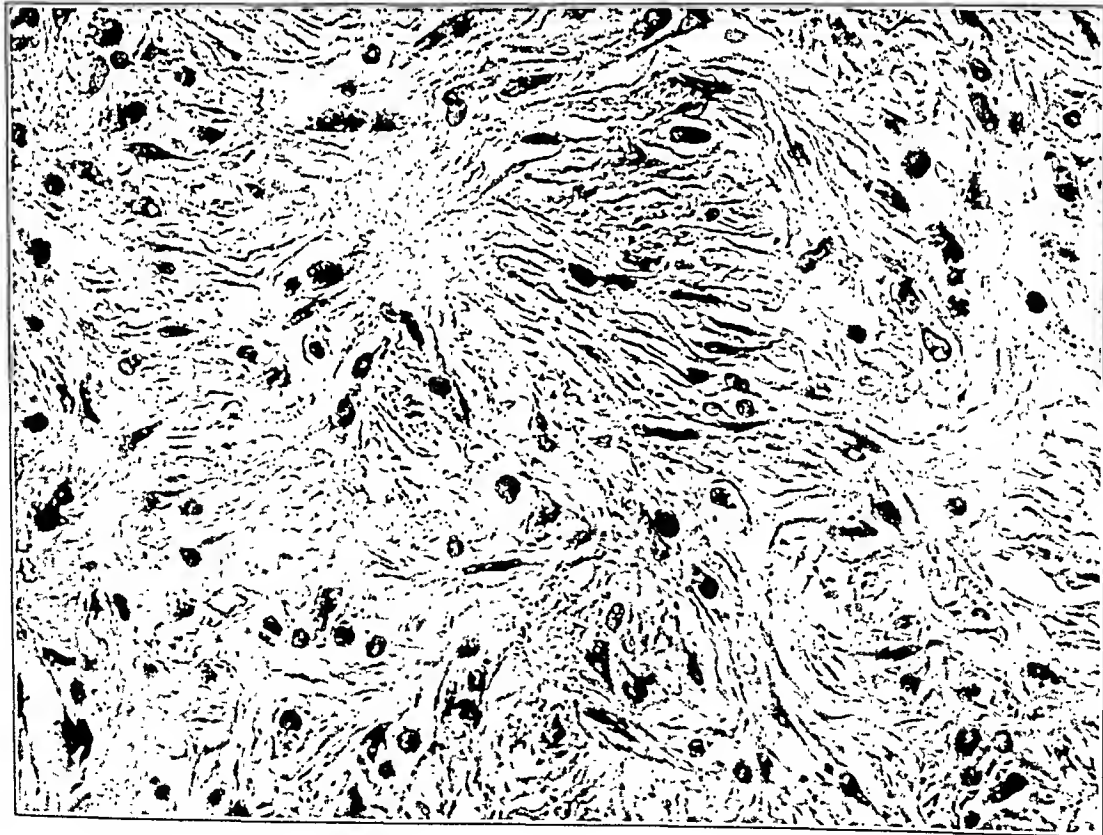
FIG. 14. Scirrhou Hodgkin's lesion in lymph nodes. Giant cells with pseudopod to left.  $\times 800$ .

FIG. 15. Sternberg giant cell free within alveolar space of lung.  $\times 800$ .

FIG. 16. Complex mitotic figure in Sternberg giant cell in lymph node.  $\times 1000$ .



11



12

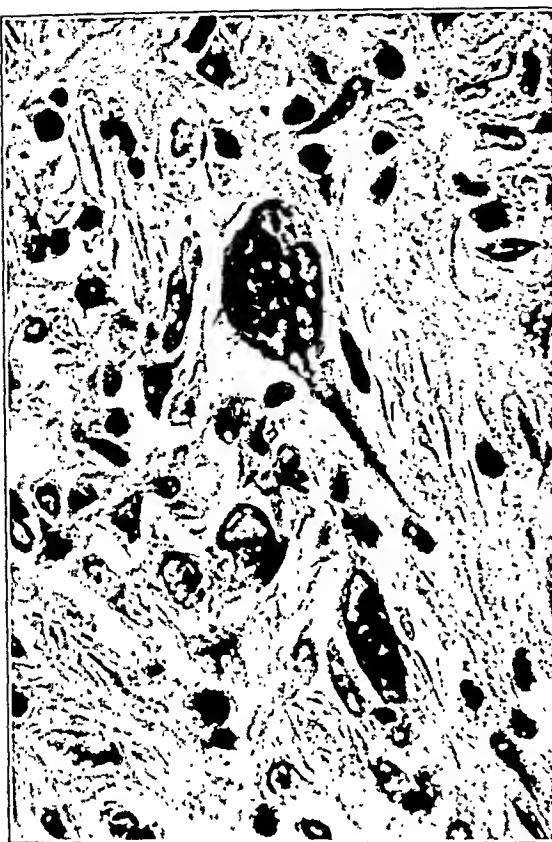
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Nature of Hodgkin's Disease

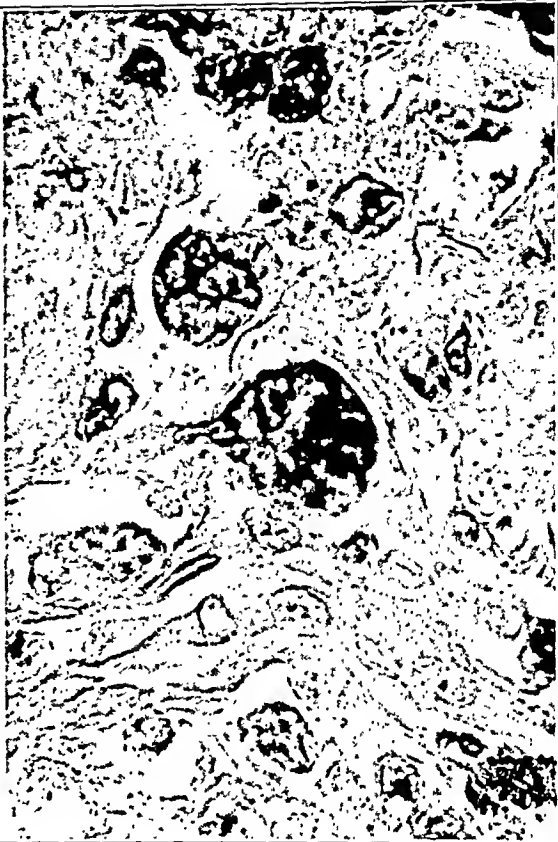
PLATE 122

FIG. 17. Large lymph sinus in lymph node filled with Hodgkin's tumor cells.  
Note pleomorphism of cells.  $\times 1000$ .

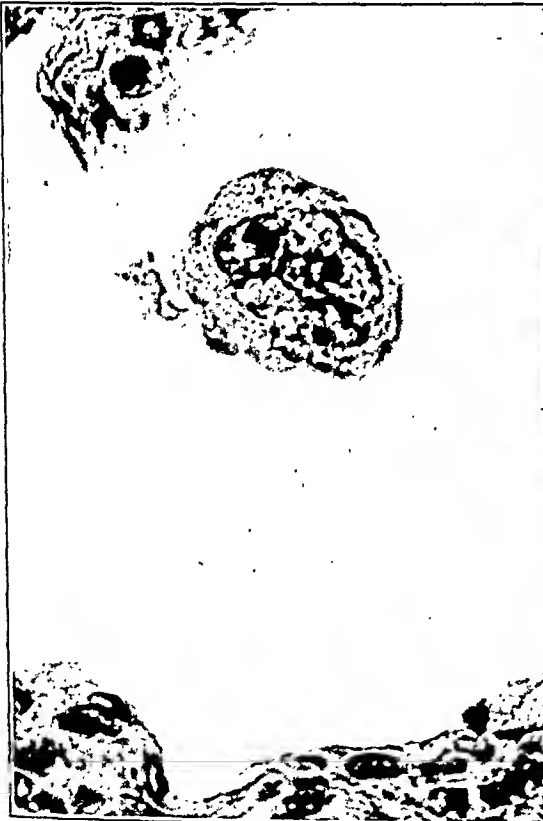
FIG. 18. Small area from same section as Fig. 17 showing a predominance of  
Sternberg giant cells.  $\times 1000$ .



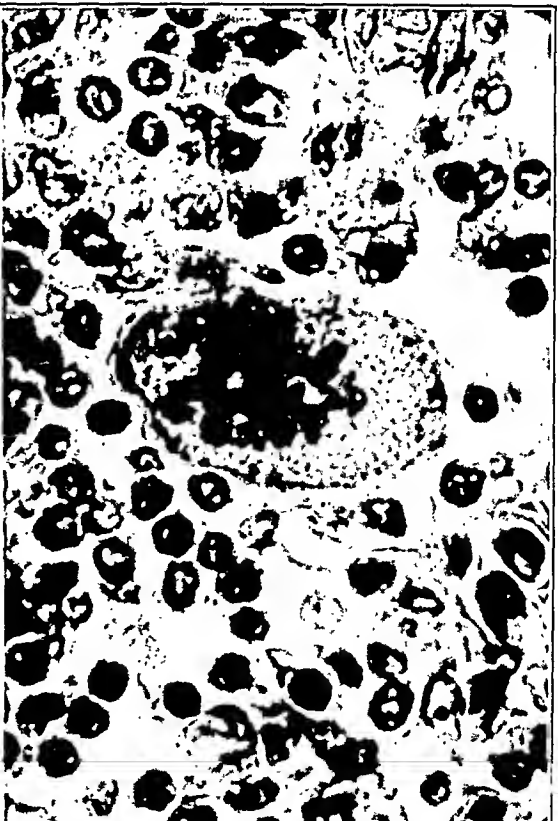
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15



16

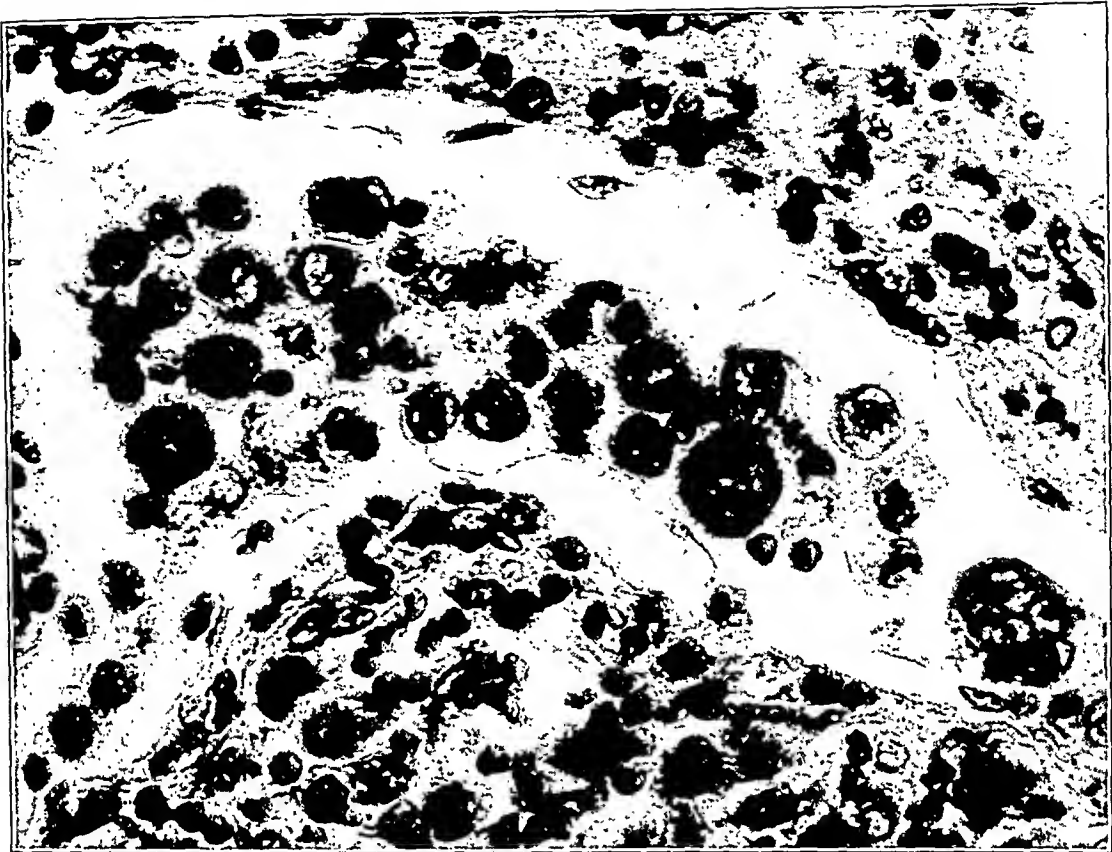
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Nature of Hodgkin's Disease

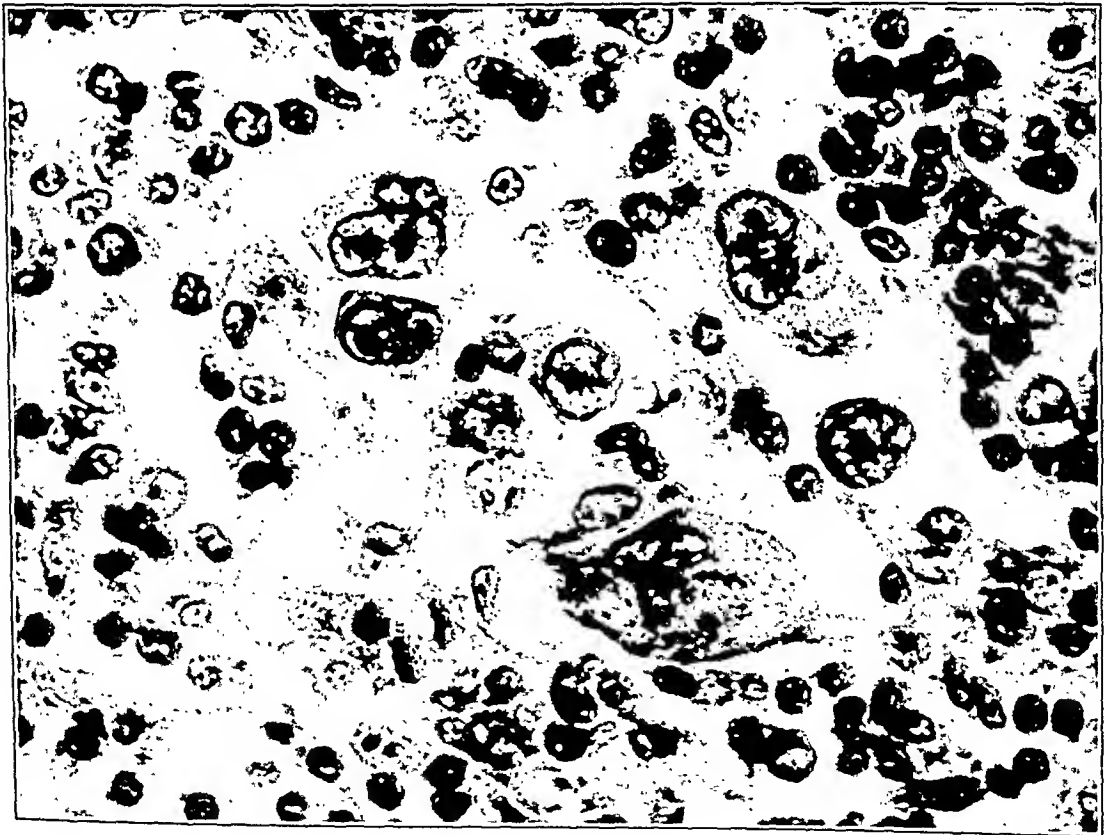


PLATE 123

- FIG. 19. Typical morphology of tumor cells in Hodgkin's disease. These cells represent the developmental cycle of the megakaryocyte. Mitosis just above center of field. Such a histopathological picture we regard as typical and as sufficient to justify a diagnosis of *Hodgkin's disease*.  $\times 1000$ .
- FIG. 20. Another area from same section as Fig. 19. Shows an area of necrotic tumor heavily infiltrated with neutrophils.  $\times 1000$ .



17

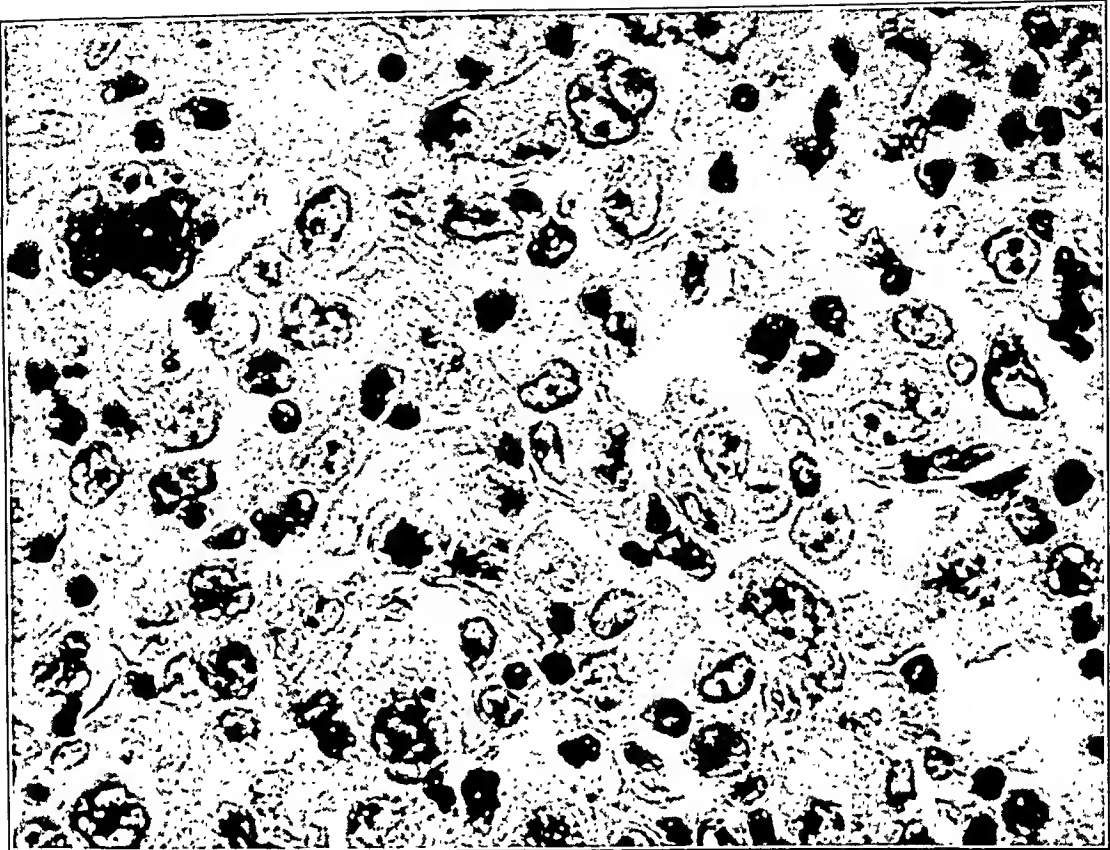


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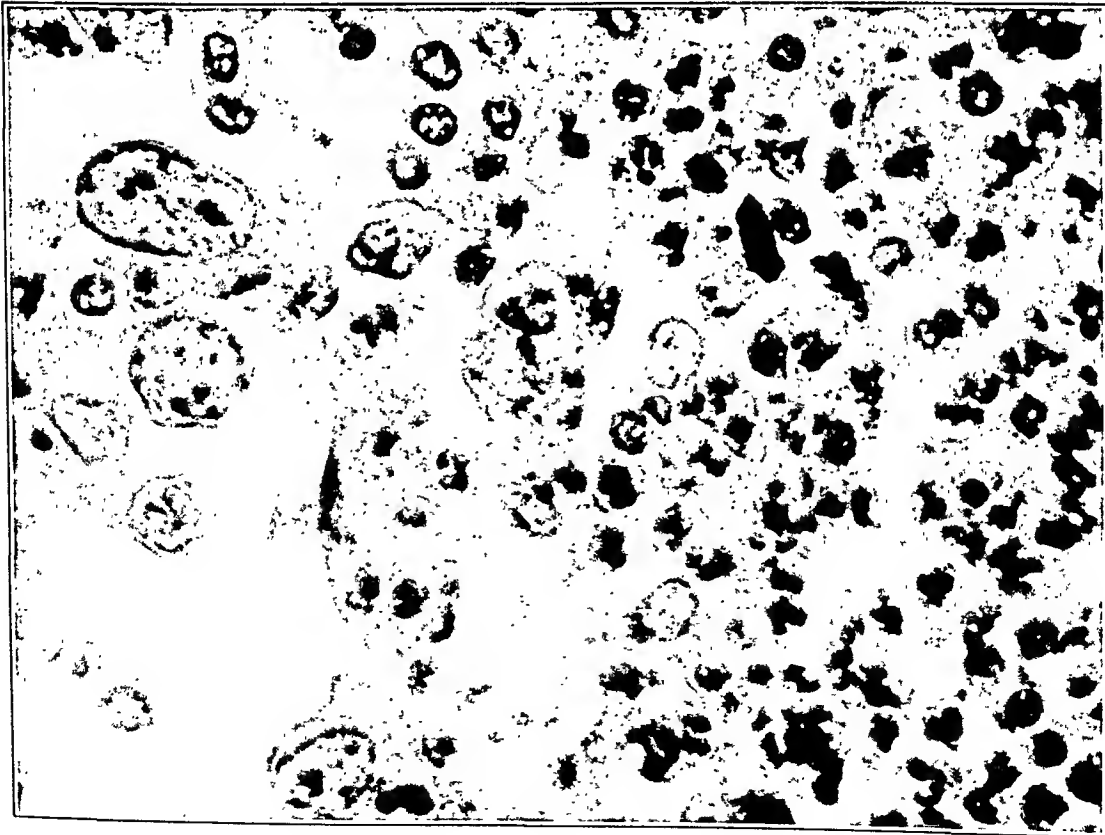
Medlar

Nature of Hodgkin's Disease





19



20

Köhler <sup>7</sup> claimed to have been able to demonstrate the organism by the use of a silver impregnation after formalin fixation and decalcification in 5 per cent nitric acid. The staining of the *Treponema pallidum* requires a difficult and exacting technique, and decalcification in 5 per cent nitric acid renders the technique very uncertain. Römer <sup>10</sup> has observed a degeneration of the enamel organ in congenital syphilitics which he thought probably was due to the direct invasion of spirochetes, but makes no reference to the finding of the organism. Josefson <sup>11</sup> believed the disturbance in calcification was due to an interference in the function of the glands of internal secretion and attributed it specifically to the thyroid gland. Because of questionable technical procedures employed by Köhler, and insufficient evidence presented by Cavallaro, uncertainty and doubt have constantly existed.

In an attempt to settle this uncertainty, nine suspected congenital syphilitic full-term fetuses\* have been examined. The enamel organs of the central incisor and the first molar of the permanent dentition were dissected from the mandible under a dissecting microscope. These tissues, with a specimen of liver from the same fetus, were fixed in neutral 4 per cent formaldehyde. Slides were prepared according to the methods of Levaditi, Warthin-Starry, Jahnke and Giemsa. The liver and enamel organs were treated at the same time, and sections of each were mounted on the same slide. Every effort has been made to give the enamel organs and the liver identical treatment.

Spirochetes morphologically similar to *Treponema pallidum* were demonstrated in the liver of six fetuses. One hundred and fifty slides from the six proved congenital syphilitic fetuses were examined and, with the exception of one structure whose identity as a spirochete is questionable, the *Treponema pallidum* was not found within the dental tissues.

Although the staining of *Treponema pallidum* in tissues is, as a rule, difficult, the fact that they have been demonstrated in the livers of these fetuses renders it extremely unlikely that the absence of the organisms in the tooth germ can be attributed to technical difficulties. It is possible that the spirochete in the tooth germ is as

\* The author thanks Dr. David Seecof and Dr. Paul Gross for aid in securing material.

# AN INVESTIGATION OF SPIROCHETOSIS OF THE DENTAL ANLAGE IN CONGENITAL SYPHILIS \*

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The dental defects that are found clinically associated with congenital syphilis were demonstrated by Jonathan Hutchinson <sup>1</sup> and Edouard Fournier <sup>2</sup> many years ago. Since that time considerable controversy has been centered around both the significance and the cause of these defects.

Much evidence has accumulated to support the clinical observation that a definite deformity is associated with congenital syphilis. Principally due to the contributions of Cavallaro, <sup>3</sup> Stein <sup>4</sup> and Karnosh, <sup>5</sup> the morphology of these defects has been well established. Some investigators have attributed the cause of this characteristic malformation to a spirochetosis of the tooth germ. Pasini <sup>6</sup> in 1905 and Köhler <sup>7</sup> in 1913 claimed to have been able to demonstrate the *Treponema pallidum* in developing enamel organs. Cavallaro <sup>8</sup> in 1909 published an extensive work entitled, "Syphilis and its Relation to Dentition." The English translation of this work by Schaffner states "The *Spirocheta pallida* is abundantly found in the dental follicles of developing teeth," whereas the original article states, "*La Spirochaeta pallida* s'e trovata costantemente assente, sia nel follicolo dentario, sia nella polpa dentaria, sia nel periostio alveolo-dentale di soggette eredo-sifilitici." This we interpret as, "The *Spirocheta pallida* are constantly absent in dental follicle, the dental pulp and alveolo-dental periosteum in subjects with congenital syphilis." In 1925 Cavallaro <sup>9</sup> published "Le Sindromi Sifilitiche Boccali" in which he states that his earlier conclusions were based upon the staining technique of Hoffman, but later by the use of the May-Grünwald-Giemsa stain he had been able to find the organisms, as had Pasini at a previous date. Cavallaro's article of 1925 includes a drawing of the dental pulp with spiral organisms within the blood vessel wall.

\* Received for publication April 18, 1931.



difficult to stain as those in acquired syphilis and that six cases are insufficient to exclude their presence.

Because the *Treponema pallidum* is more commonly found in and about the wall of blood vessels, one would expect that occasional organisms might be found in the dental pulp, but not within the avascular enamel organ. Hypoplasia of the enamel can not be explained as the result of spirochetosis unless the organisms can be found intimately associated with the areas of degeneration which have been demonstrated within the developing enamel organ in congenital syphilitic fetuses.

The results of this investigation indicate that there is insufficient evidence to justify the conclusion that the characteristic congenital syphilitic dental deformities are the direct result of the invasion of the enamel organ by the *Treponema pallidum*.

I wish to express appreciation to Dr. Howard T. Karsner for his aid and advice in this work.

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Using a larger series of cases, the following results were obtained. Of 100 specimens obtained at autopsy fifty-four contained a fold or folds of mucous membrane, polypoid in nature, protruding through the orifice of the duodenal papilla and apparently partially obstructing the opening. In size and shape these polyp-like structures varied from one with a single knob to one which protruded 3 mm. beyond the orifice and which was divided into several parts; each part was definitely knob-like at the extremity. In thirty-two of these fifty-four specimens there was definite congestion around the orifice of the papilla, and occasionally in the projecting mass, suggesting a definite inflammatory process.

In every specimen in which congestion of the orifice was found there was also swelling of the wall of the papilla in this region (Fig. 1). The congestion, however, was never found to extend more than 3 or 4 mm. along the intestinal surface of the papilla.

On opening these specimens along the longitudinal axis, avoiding cutting the growth at the orifice, it was found that the folds in the ampullary portion of the common bile duct were prominent and that where congestion was present they were covered with thick mucus. Also, the polypoid character of the masses in the orifices was emphasized by enlargement or swelling of the tips of these folds (Figs. 2 and 3).

In the specimens in which the common bile duct and the pancreatic duct each had a separate opening at the outlet of the papilla, these swollen folds at the orifice were confined to the common bile duct and such change was not present in the pancreatic duct. In those cases in which the ducts united 1 to 2 mm. or more from the orifice of the papilla these swollen folds were always on the side of the common bile duct. Microscopic study of these folds, using hematoxylin and eosin, Van Gieson's connective tissue stain and the mucicarmine stain, demonstrated these structures to be composed of dilated acini filled with thick mucus (Fig. 4). Each fold contained several of these acini varying in size. Their epithelial lining was composed of tall, columnar cells on a thin basement membrane of connective tissue. The cytoplasm of the cells contained mucus confined in the free end of the cell. The nuclei, as a rule, were confined to the bases of the cells and were fairly large, lightly staining vesicular structures. Other acini contained a small amount of thin mucus and short columnar cells with a small amount of cyto-

## INFLAMMATORY ADENOMATOID HYPERPLASIA OF THE MAJOR DUODENAL PAPILLA IN MAN \*

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It is generally admitted that inflammatory adenomatoid hyperplasia is of widespread occurrence in the human body. In such locations as the mucous membrane of the gastro-intestinal tract, uterus and nares it produces diffuse overgrowth, or single or multiple polypoid excrescences which constitute definite clinical entities, and are a feature of the late stages of chronic catarrhal inflammation of these tissues. The process affects both glands and stroma. The glands become hypertrophic or cystic and the lining cells are increased in size and number. The stroma shows new and tortuous blood and lymph vessels, multiplication of stroma cells and infiltration by lymphocytes. The process tends first to produce a diffuse thickening, but focal overgrowth of the tissue, edema, venous stasis and mechanical traction lead to papillary and polypoid outgrowths which may become numerous and bulky. In this condition there is a tendency to further overgrowth of the predominating element, glands, stroma or vessels. In the nares the mucous polyps show wide variations in structure, some being glandular, others myxomatous, and still others angiomatous, but the tendency to true tumor growth is slight.

In a recent anatomical study of the major duodenal papilla in man it was noticed that in a series of forty-eight specimens obtained at autopsy, twenty-three contained what appeared to be a polypoid structure in the outlet (Figs. 1 and 2).

Such a high frequency of occurrence aroused further interest and justified further study, especially when the literature failed to reveal any evidence that this condition has received due recognition. Letulle and Nattan-Larrier,<sup>1</sup> however, mention the occasional projection of the ends of the longitudinal mucous folds of the ampulla through the outlet.

\* Received for publication May 1, 1931.

bladder were present in five, and ulcer in the stomach or duodenum in eleven.

The most outstanding fact in the abnormality of the papilla is the sex incidence. In the fifty-four cases in which this condition was present thirteen patients were women and forty-one men. This bears the same proportional relation to sex as carcinoma in this region.

The age at which this change was found ranged from 36 to 96 years. Most of the patients, however, were in the fourth, fifth and sixth decades.

### SUMMARY

1. In 54 per cent of 100 cases in which autopsy was performed, definite changes were found in the major duodenal papilla. The changes apparently were benign and did not cause definite symptoms.

2. Enlargement of the ends of the folds and thickening of the papillary wall were due to the presence of hyperplastic mucous glands, resulting probably from repeated attacks of inflammation at the outlet of the papilla.

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### DESCRIPTION OF PLATE

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#### PLATE 124

FIG. 1. The major duodenal papilla. There is a polypoid structure in the orifice, and thickening of the wall. The orifice does not appear to be occluded in this particular specimen.

FIG. 2. Outlet of the major duodenal papilla. The swollen end of one fold containing numerous mucus-secreting acini is seen. The framework of this swelling is dense connective tissue. There is definite thickening of the wall of the papilla on both sides. This is also due to dilated glandular acini, as can be seen in the picture: "a" common bile duct, "b" pancreatic duct.

FIG. 3. Interior of the major duodenal papilla. The longitudinally coursing folds of the mucosa are seen.

FIG. 4. Higher power of Fig. 3 through the swollen end of one of the folds in the outlet of the papilla to show the greatly dilated acini. Hematoxylin and eosin stain.  $\times 150$ .

plasm, which sometimes contained mucus. The nuclei were small and heavily stained. No two acini appeared to have the same shape, the same amount of mucus, or to be composed of cells of similar size.

As has been mentioned, in the specimens in which definite congestion was present there was also enlargement of the wall of the papilla at the outlet. On section, this thickened wall was found to contain dilated acini similar to those in the swollen ends of the folds, and collections of lymphocytes on the surface and in the connective tissue.

The presence of these swollen folds in the orifice of the ampulla was suspected of producing some obstruction to the outflow of bile, and, therefore, should produce some relative dilatation of the common bile duct due to increased pressure within the duct. Therefore, the circumference of the common bile duct was measured at the level of the union of the right and left hepatic ducts, halfway between this level and the intestine, and immediately outside of the intestine.

The average circumference of the common bile duct at the junction of the right and left hepatic ducts midway between this level and the duodenal wall, and immediately outside of the duodenal wall in the specimens with the supposed obstruction in the outlet of the papilla, were 1.6, 1.4 and 1.2 cm. respectively; while in the cases without hypertrophy and obstruction at the outlet, the measurements in the same regions were 1.5, 1.3 and 1.1 cm. respectively.

From these observations it is apparent that there is no appreciable effect on the size of the common bile duct, such as would be manifested by definite obstruction.

This might be explained by the fact that if there is any increase in intraductal pressure it is taken up by the gall-bladder.

What effect this condition has, if any, on the outflow of bile could not be determined on the dead subject, but it might possibly explain the retarded or delayed bile flow which is often encountered in the living subject.

Correlation of the changes that have been described with other changes in the body was not constant, neither was there suggestive correlation with any other associated factors. In the fifty-four cases the following more important conditions were present: hypertrophy of the prostate gland in nineteen cases; adenoma of the thyroid gland in six; polyps in the stomach, colon or uterus in thirteen; carcinoma of the colon, rectum or stomach in twelve; and leiomyoma of the uterus in four. Of these, gallstones confined to the gall-

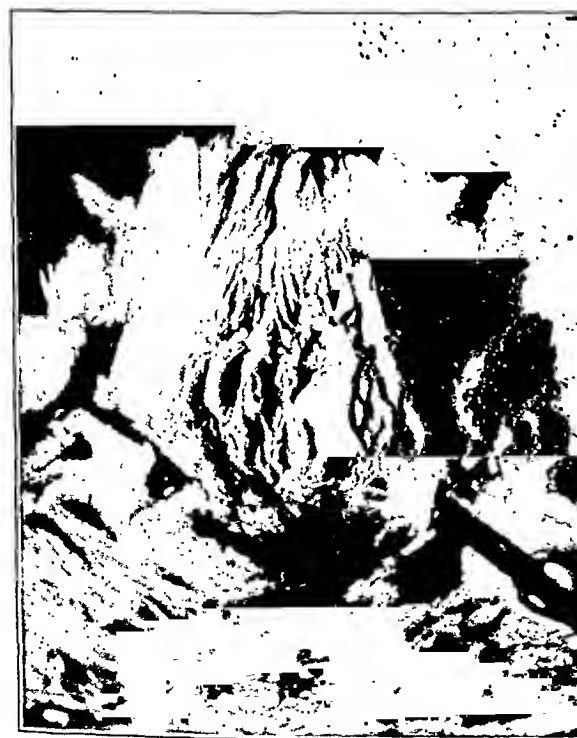




1



2



3



4

Dardinski

Adenomatoid Hyperplasia of Duodenal Papilla

Tumor I). Since this tumor is practically the only one to grow from cell-free filtrates, and since there has been considerable question as to whether this is a true tumor in the sense of mammalian tumors or whether it is due to an infectious agent or enzyme, it was thought that such an experiment might shed some light on this much disputed subject.

## EXPERIMENT I

### *Methods and Materials*

The tumor used in these experiments was obtained through the courtesy of Dr. H. B. Andervont of the Harvard Medical School. The tumor supplied us was finely minced, taken up in a small amount of Locke's solution and injected intramuscularly into a young Plymouth Rock hen. After ten days the tumor had grown to a large size. The hen was killed and the tumor removed sterily. It was then finely minced with scissors, passed through a food chopper and ground in a sterile mortar with sand. One portion of this tumor mass was diluted to a 1:5 suspension with Locke's solution and was called "tumor mash." The remainder was diluted 1:10 with Locke's solution, centrifuged, and the supernatant fluid passed through a Berkefeld N filter. This material was called "tumor filtrate."

Testicle extract was freshly prepared from testes of normal, healthy, adult rabbits. The testes were removed aseptically,\* the fat was dissected off, and they were finely minced and ground with sterile sand. Sufficient Locke's solution was added to make a 1:10 suspension. This material was centrifuged at high speed for fifteen minutes and the supernatant fluid was passed through a Berkefeld N filter. This filtrate was tested for sterility before use.

Twelve Plymouth Rock chickens, 8 weeks old, were used in the experiment, each chicken receiving four intradermal inoculations in the skin of the breast. Before inoculation, mixtures consisting of equal parts of tumor mash and saline, tumor mash and testicle extract, tumor filtrate and saline, and tumor filtrate and testicle extract were prepared sterily. The scheme of inoculation was as follows: upper left area received 0.5 cc. of the tumor mash-saline emulsion; upper right area received 0.5 cc. of the tumor mash-testis extract emulsion; lower left area received 0.5 cc. of the tumor

\* All operations on animals were carried out under full ether anesthesia.

# THE EFFECT OF TESTICLE EXTRACT ON THE ROUS SARCOMA \*

DONALD C. HOFFMAN, M.D., FREDERIC PARKER, JR., M.D., AND  
THOMAS T. WALKER, M.D.

(From the Pathological Laboratory, Boston City Hospital, Boston, Mass.)

In recent publications, Duran-Reynals<sup>1, 2, 3</sup> has shown that there exists in the testes of normal rabbits a substance, extractable in water or saline, which has the remarkable power of greatly enhancing skin lesions due to vaccine virus and to staphylococcus. This finding was confirmed by one of us<sup>4</sup> working with vaccine virus and extending the work to determine the effect of this substance on the viruses of herpes, vesicular stomatitis and Borna disease. In each case the resultant infection was greatly enhanced. McClean,<sup>5</sup> working with vaccine virus, reported similar results. Pijoan<sup>6</sup> was able to enhance the lesions produced by twenty different bacteria by means of this substance. Ledingham and Barratt<sup>7</sup> have referred to this enhancing agent as the "Reynals' factor." Hoffman and Duran-Reynals<sup>8</sup> reported the fact that this extract failed to enhance the lesions produced by certain toxins and enzymes.

All the references thus far are concerned solely with the effect of testicle extract upon infections, toxins and enzymes. As to the effect of this substance on cell activity, there have been references to the stimulation of cell growth *in vitro* by testicle as well as other organ and tissue extracts.<sup>9, 10, 11</sup> The effect of testicle extract on cell behavior *in vivo* is referred to in papers by Aievoli,<sup>12</sup> and Rho,<sup>13</sup> who reported a stimulation of healing in chronic ulcers by means of such a substance.

On the other hand, Duran-Reynals<sup>14</sup> recently reported that the Brown-Pearce rabbit tumor was greatly inhibited by incubating the tumor cell suspension with testicle extract at 37° C for from two to three hours prior to inoculation. This is the only reference that can be found dealing with the effect of testicle extract on tumor growth.

It was felt, therefore, that it would be of considerable interest to carry out an experiment to determine what effect, if any, this enhancing substance would have upon the Rous sarcoma (Chicken

\* Received for publication July 18, 1931.



plus saline. This is, of course, only an approximate figure and, if anything, shows less than the real enhancement phenomenon.

TABLE I  
*Description of Tumor Nodules 14 Days After Inoculation*

Chicken No.	Tumor mash 0.25 cc. plus Saline 0.25 cc.	Tumor fil- trate 0.25 cc. plus Testicle ext. 0.25 cc.	Tumor filtrate 0.25 cc. plus Saline 0.25 cc.	Tumor filtrate 0.25 cc. plus Testicle ext. 0.25 cc.
	cm.	cm.		
1	1.5 X 1.7	2.3 X 2.0	3 small nodules	multiple nodules
2	3.0 X 1.5	6.0 X 4.0	few small nodules	multiple nodules filling marked area
3	3.5 X 2.0	5.0 X 4.0	few superficial and discrete nodules	same as No. 2 but ex- tending subcutaneously
4	1.5 X 1.3	2.5 X 2.0	few small discrete nodules	several small nodules
5	1.5 X 1.0	1.5 X 1.2	1 small subcutaneous nodule	1 small subcutaneous nodule
6	2.0 X 1.0	2.5 X 2.0	3 small nodules, larg- est 0.4 cm. diam.	several small nodules, largest 0.5 cm. diam.
7	3.5 X 2.0	5.0 X 4.0	few discrete nodules	multiple nodules filling marked area
8	2.0 X 1.7	5.0 X 4.0	few localized nodules	multiple small nodules
9	1.5 X 1.3	2.0 X 2.0	1 small nodule	multiple small nodules filling marked area
10	3.5 X 1.8	5.5 X 3.5	few localized nodules	multiple small nodules
11	2.0 X 1.5	3.5 X 4.0	1-2 small nodules	several nodules
12	1.5 X 1.2	2.5 X 2.0	2 small nodules	multiple nodules
Average area in square cm.	3.5	11.8	..	..

On the other hand, the tumors resulting from injection of tumor filtrate consisted in almost every case of several small nodules, either discrete or confluent. A fair measurement of these was not possible. Suffice it to say that those nodules occurring following injection of

filtrate-saline suspension; and lower right area received 0.5 cc. of the tumor filtrate-testis extract suspension. Each inoculated area was marked off by painting a square about 2.5 by 2.5 cm. to 3 by 3 cm. around the site of injection with an alcoholic solution of gentian violet.

It was noted at the time of the inoculations that those mixtures containing testicle extract spread through the skin extremely rapidly, whereas those wheals containing only saline persisted for a considerable length of time.

### *Results*

Four days after inoculation of the materials the chickens were examined for the first time. At that time, in each case, small tumor nodules had begun to form in the lower left and lower right regions, where tumor mash had been injected with testicle extract, and its control containing the same amount of mash plus saline. In each case except one, the tumor nodule resulting from the injection of mash plus testicle extract was from one-and-a-half to ten times as large as the corresponding control lesion. The areas which had received tumor filtrate had not shown tumor growth at that time. The tumors were measured every two days thereafter, and each time the areas which had received testicle extract in the inoculum showed much larger tumors than the controls. Those which had received tumor filtrate showed tumors for the first time ten days after inoculation. In each case with these tumors, the regions which had received testicle extract showed multiple small nodules which covered a considerably larger area than the corresponding controls. The fourteenth day after inoculation was the last day on which all chickens were still alive. The individual measurements and descriptions of the various tumors are recorded in tabular form in Table I.

It can be seen from the table that in each case the tumor resulting from the injection of mash plus testicle extract was larger than the corresponding control—in many cases markedly so. These tumors were rapidly growing and formed one nodule which was easy to measure in its two diameters. The approximate plane area of each nodule was determined and the average calculated for all the chickens. Based on average areas, the tumors resulting from mash plus testicle extract were 3.37 times as large as those arising from mash

As in the first experiment the injection masses were prepared before inoculation as follows: mixtures of equal parts of tumor filtrate and Locke's solution were made in one tube, equal parts of tumor filtrate and rooster testicle extract in another, equal parts of tumor filtrate and rabbit serum in another, and equal parts of tumor filtrate and rabbit testicle extract in the last.

Ten healthy young Plymouth Rock roosters, 8 weeks old, were used. Each received four intradermal injections according to the following scheme: upper left area, 0.5 cc. of the filtrate-Locke's solution mixture; lower left area, 0.5 cc. of the filtrate-rooster testicle extract mixture; upper right area, 0.5 cc. of the filtrate-rabbit serum mixture; and lower right area, 0.5 cc. of the filtrate-rabbit testicle extract mixture.

At the time of the inoculations it was observed that the mixture which contained rabbit testicle extract spread very rapidly through the tissues, whereas the other three mixtures left a wheal for an appreciable length of time.

### *Results*

The first observations were made eight days after inoculation. By this time every chicken had developed tumors in each area. In each instance but one, the tumors growing after inoculation of filtrate plus rabbit testicle extract were considerably larger than the Locke's solution control. In the other areas no such constant difference in the size of the tumor nodules was apparent. In practically every case the control area was the smallest.

The tumors were measured at two-day intervals and the thirteenth day after injection was the last day when all animals were still alive. The tumors were growing rapidly, and this day was selected for recording the measurements in tabular form. These appear in Table II.

As in the first experiment, the tumors resulting from the injection of tumor filtrate plus rabbit testicle extract were in each case larger than the Locke's solution control tumors. In addition they were also larger than the rooster testicle extract and rabbit serum tumors. In want of a better method of getting an average size for comparison, the plane areas were again computed and averaged. As can be seen from the table the average area of the tumors resulting from injection of tumor filtrate plus rabbit testicle extract was 12.67 square

tumor filtrate plus testicle extract, showed approximately the same degree of enhancement as the ones growing from tumor mash. The striking feature was the development of a multiplicity of small confluent nodules in the extract areas which was never observed in the case of the nodules arising from tumor filtrate plus saline.

After the death of the chickens, autopsies were performed on each and bits of the skin nodules were fixed in alcohol-formalin. Celloidin sections were made from each nodule and stained with hematoxylin and eosin. Each section showed typical Rous sarcoma, but no differences histologically could be made out in the various tumors. Most of the chickens showed metastatic nodules in lung and liver.

Thus it was seen that there had occurred marked enhancement in the growth of these tumors when rabbit testicle extract had been used as a diluent for the tumor mash or filtrate.

However, there seemed to be a possibility that this enhancement might have resulted either wholly or in part from the presence of foreign protein (rabbit protein) rather than the testicle extract principle itself. Also, the question arose as to whether extract of rooster testis would exercise the same effect as rabbit testis. Consequently, a second experiment was designed to clarify these points.

## EXPERIMENT II

### *Methods and Materials*

The chicken tumor used in the second experiment was likewise procured fresh from Dr. Andervont at the Harvard Medical School and inoculated into the breast muscle of a young Plymouth Rock hen. After ten days, the hen was killed and the tumor removed aseptically. The procedure for preparing the tumor injection material was the same as in the first experiment with the exception that no tumor mash was used and the filtrate dilution was 1:5.

The same rabbit testicle extract was used as in Experiment I.

Rooster testicle extract was made in exactly the same manner as rabbit testicle extract.

Normal rabbit serum was obtained by bleeding a normal rabbit from the heart and collecting the blood in a sterile tube. After it had clotted and the clot separated, the tube was centrifuged at high speed for twenty minutes and the serum was pipetted off.

## DISCUSSION

The experiments here reported show clearly that rabbit testicle extract enhances quite markedly the growth of the Rous sarcoma (Chicken Tumor I), following its injection together with the tumor-producing agent. This enhancement occurs equally well following inoculation of cell-free filtrate of the tumor or with injection of a mash consisting of tumor cells in suspension. In the latter case the resultant tumors appeared much more quickly after inoculation, but the degree of enhancement was essentially the same as that following injection of the filtrate. The tumors arising from tumor mash plus rabbit testicle extract, using the average area of the tumors on the fourteenth day, were 3.37 times the average size of the tumors resulting from injection of tumor mash plus saline. The tumors which resulted from injection of the Berkefeld filtrate plus rabbit testicle extract based on average areas on the thirteenth day were 3.25 times the size of those arising from filtrate plus Locke's solution. Thus it will be seen that the degree of enhancement using rabbit testicle extract was practically the same in two different experiments using tumor mash in one instance and cell-free filtrate in the other.

An interesting finding was the fact that rooster testicle extract prepared under exactly the same condition as rabbit testicle extract was wholly without this enhancing property. Just why this should be true is not clear and we shall not attempt to offer an explanation. However, it was noted at the time of inoculation that the wheals caused by intradermal injections disappeared surprisingly rapidly when rabbit testicle extract was a part of the injection mass, whereas the wheals persisted for a considerable length of time when rooster testicle extract was used.

That normal rabbit serum is not responsible for this enhancement is clearly shown in the second experiment. There was a slight degree of enhancement, as indicated by the average tumor sizes on the thirteenth day. Those tumors which arose from tumor filtrate plus rabbit serum were 1.3 times the size of the Locke's solution control tumors. This is in marked contrast to the enhancement of the tumors by rabbit testicle extract, which not only increased in two plane dimensions, but often protruded and practically always invaded the deep tissues.

cm., whereas the Locke's solution control tumors averaged 3.9 square cm. The rooster testicle extract tumors averaged 3.2 square

TABLE II

*Description of Tumor Nodules 13 Days After Inoculation*

Chicken No.	Tumor filtrate 0.25 cc. plus Locke's sol. 0.25 cc.	Tumor filtrate 0.25 cc. plus Rooster testicle ext. 0.25 cc.	Tumor filtrate 0.25 cc. plus Rabbit serum 0.25 cc.	Tumor filtrate 0.25 cc. plus Rabbit testicle ext. 0.25 cc.
	cm.	cm.	cm.	cm.
13	2.5 X 2.0	3.0 X 2.0	2.6 X 3.0	5.0 X 3.5
14	1.5 X 1.3	0.9 X 1.0	1.7 X 2.1	deep extension 5.0 X 5.0
15	3.7 X 2.0	3.0 X 1.4	2.4 X 1.9	deep extension 6.4 X 2.3
16	2.8 X 2.0	2.8 X 1.8	2.7 X 2.5	4.0 X 2.7
17	0.4 X 0.4	1.4 X 0.4	2.2 X 2.2	deep and diffuse 5.5 X 2.8
18	3.2 X 1.5	2.3 X 1.4	3.0 X 3.0	deep extension 3.5 X 4.3
19	0.5 X 0.5	0	1.0 X 1.0 0.2 X 0.2	3.0 X 1.7
20	1.5 X 1.3	2.5 X 1.2	7 confluent nod- ules, largest 0.7 X 0.6	large confluent nod- ules, deep exten- sion 2.5 X 1.8
21	2.5 X 2.1	2.8 X 2.0	2.8 X 2.8	ulcerated 4.0 X 3.2
22	2.0 X 1.9	2.5 X 1.4	deep extension 2.3 X 1.7	deep extension 4.0 X 2.5
Average area in square cm.	3.9	3.2	5.05	12.67

cm., and the rabbit serum tumors averaged 5.05 square cm. It would seem, therefore, that the mere presence of rabbit protein produced a considerable enhancement of the tumors, but not nearly so strikingly and constantly as did the rabbit testicle extract.

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It is beyond the scope of this brief communication to attempt to theorize about the mechanism of this remarkable phenomenon. We merely present the experimental facts. For a discussion of this subject, the reader is referred to recent publications by McClean<sup>5</sup> and by Hoffman and Duran-Reynals.<sup>8</sup>

The question naturally arises as to whether this tumor behaves like a true tumor in the sense of mammalian tumors, or whether it is the manifestation of a virus disease, an infectious granulomatous process, or an enzyme-like substance. Without attempting to make any statement which would align us on one side or the other of this controversy, we merely point out the fact that the tumor is greatly enhanced by this agent, which, so far as we can say at present, enhances only virus or bacterial infections. That this tumor is not due to a bacterial agent is pretty well accepted at this time. Enzymes are apparently not enhanced by this substance, and the only experimental work on mammalian tumors thus far reported is that by Duran-Reynals<sup>14</sup> who showed that a rabbit tumor of epithelial origin is actually inhibited by this substance. It is only fair, however, to point out that in the experimental work of Duran-Reynals on the rabbit tumor, the tumor mash plus testicle extract was incubated for from two to three hours at 37° C before injection. This technique was not followed by us. However, our injection masses were made up approximately one to two hours before inoculation and stood at room temperature during that time.

Thus there may or may not be evidence hinting that this tumor falls in the class of diseases due to infectious agents. Before we are definitely willing to say it is so, we should like to see considerable more work done on the effect of this enhancing agent.

### CONCLUSIONS

1. Rabbit testicle extract markedly enhances growth of the Rous sarcoma (Chicken Tumor I) in chickens.
2. This effect is the same whether tumor mash or a cell-free filtrate of the tumor is used in the inoculations.
3. Rooster testicle extract causes no enhancement.
4. Normal rabbit serum causes a slight degree of enhancement.





SCIENTIFIC PROCEEDINGS OF THE  
THIRTY-FIRST ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS AND  
BACTERIOLOGISTS

CLEVELAND, OHIO

April 2 and 3, 1931

George Shanks,  
Walter J. Siebert,  
Louis Charles Simard,  
M. Maxim Steinbach,  
Max Strumia,  
Kornel L. Terplan,

Richard Thompson,  
Thomas T. Walker,  
Isabel Mary Wason,  
Theodore R. Waugh,  
Emil Weiss,  
Harry Zimmerman.

Voted to accept the resignations of Dr. O. H. P. Pepper and Dr. Harry C. Solomon.

Voted to record with regret the deaths of Dr. Henry Albert, Dr. C. A. Hamann, Dr. C. A. Krumweide and Dr. R. W. Webster.

Voted to adopt as the topic for the symposium for 1932 the subject of Tuberculosis.

The Secretary drew attention to the difficulties that have to do with selection of titles for the program and it was a general agreement that in the notice of call for the annual meeting it be stated that abstracts must accompany the title cards, drawing to the attention of contributors that wherever possible these abstracts should be prepared for publication with the understanding that if the contributor desires to make any change in the abstract he may do so by providing a substitute abstract before the meeting.

The Secretary drew attention to the demand for publicity on the part of Science Service and newspaper reporters. It was suggested without any definite action being taken that members contributing papers be requested to contribute material suitable for publicity purposes.

The meeting for next year will be held in Philadelphia on April 28 and 29, 1932, which is the week preceding the meetings of the Association of American Physicians in Atlantic City.

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND  
BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

Voted to elect the following officers for 1931-1932:

<i>President</i>	WARD J. MACNEAL
<i>Vice-President</i>	E. T. BELL
<i>Treasurer</i>	FRANK B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	WILLIAM BOYD

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ABSTRACT OF MEETING OF THE COUNCIL

Voted to elect the following new members:

Carl W. Apfelbach,	Ivan C. Hall,
Granville A. Bennett,	William B. Hawkins,
Everett L. Bishop,	C. Alexander Hellwig,
Eugene A. Case,	Joseph Kasper,
Orren D. Chapman,	George J. Kastlin,
Benjamin E. Clarke,	Ralph E. Knutti,
E. V. Cowdry,	K. Robert Koch,
Gilbert Dalldorf,	George M. Lawson,
Joseph R. D'Aunoy,	Pablo Morales-Otero,
Israel Davidsohn,	Lawrence R. Morrison,
Isaac H. Erb,	E. G. D. Murray,
Sidney Farber,	T. F. Nicholson,
Clement C. Fenton,	Charles T. Olcott,
H. W. Ferris,	Isadore Pilot,
Donald T. Fraser,	Jacob Rabinovitch,
Istvan Gaspar,	Nathan Rosenthal,
Raymond H. Goodale,	James F. Rinehart,
Leonard W. Goss,	George J. Rukstinat,
Allen Graham,	Helen May Scoville,
Emmerich von Haam,	Beatrice Carrier Seegal,

markedly anticomplementary. Both the lytic and anticomplementary properties were reduced in the presence of serum. The anticomplementary action of cephalin paralleled closely its coagulative activity.

THE QUANTITATIVE DETERMINATION OF THE FIXATION OF COMPLEMENT BY IMMUNE SERUM AND ANTIGEN. Elizabeth Maltaner (by invitation), Albany, N. Y.

*Abstract.* The conditions essential to quantitative measurement of the changes in hemolytic activity of complement which take place in complement-fixation tests with immune serum were investigated, together with the relations which exist between these changes and the concentration of immune serum.

The previous observations of Morse that the point of 50 per cent hemolysis is the most exact index of the hemolytic unit of complement was applied to this work; also the findings of Brooks which demonstrated that changes in complement activity must be measured at points of equal degree of hemolysis.

The limits of concentration of reagents and the conditions under which quantitative determinations of changes in hemolytic activity could be made were established, using tubercle antigens and their specific antisera. As a result, it became apparent that the variations in fixability of different complements rendered impossible the direct measurement of the immune reaction by use of a hemolytic unit of complement. However, a constant relation was discovered between the extent of the changes in the hemolytic activity of complement and the quantities of immune serum present, which made possible the use of a "fixability unit" in determining the specific activity of serum, instead of the hemolytic unit, thus eliminating the error due to differences in the fixability of different complements.

The observations suggest that by the use of "fixability units," determined with an immune serum selected as a standard, it may be possible to measure quantitatively with different complements the activity of various other sera by comparison.

The development of a quantitative method based upon comparison with a standard immune serum should be an important step toward the standardization of the complement-fixation test, since it would permit the adoption of a specific serum as a universal standard of comparison in evaluating reactions, just as standards of this kind have been adopted in other fields, for example, in comparing toxins and antitoxins.

LOCAL ORGAN HYPERSENSITIVENESS. A NEW METHOD FOR ITS PRODUCTION IN THE RABBIT EYE. Beatrice Carrier Seegal and David Seegal (by invitation), New York City.

*Abstract.* The observations of Auer, Opie, Hanger and Menken have shown that when a local area of the body is subjected to a sterile inflammation, a foreign dye or protein circulating in the blood is concentrated in this area of local inflammation. Utilizing this principle, we have been able to show that if a rabbit's eye is inflamed and egg white is injected intravenously at the same time, the egg white is concentrated at the site of this inflammation in sufficient quantity to render this eye sensitive to subsequent intravenous injection of the homologous antigen.

The experiments were carried out on three groups of animals. Group I were the experimental animals, Groups II and III the control animals. Group I re-

# AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

COMPARISON OF KAHN, SIGMA AND HINTON TESTS. David L. Belding and (by invitation) Julia Arrowood, Boston, Mass.

*Abstract.* The Kahn, Sigma and Hinton tests were compared in respect to sensitiveness and freedom from variation in the detection of syphilitic reagin. A single lot of high titer syphilitic ascites fluid, which remained stable during the experiment, eliminated any quantitative variation in syphilitic reagin. Graded dilutions of this fluid enabled the results of each test to be expressed quantitatively in arbitrary units. As far as possible, the different antigens were prepared from the same material.

In detecting small amounts of syphilitic reagin, the Hinton test gave a positive reaction with one-half the amount necessary for a positive reading with the Kahn test and one-quarter that for the Sigma test.

The best results were obtained when each test was done with its special antigen. The acetone-insoluble Sigma extract gave similar results to the Kahn-Hinton extract which was not treated with acetone. The Hinton test was effective only with the Hinton antigen. The Kahn and Sigma tests both gave positive results with each other's antigen.

All three tests showed daily variations which appeared to be due largely to the preparation of the antigen dilution. The Hinton test showed the least fluctuation and in this respect would seem to be the most suitable for a quantitative technique.

## *Discussion*

(Dr. Reuben L. Kahn, Ann Arbor.) I would only add that one curve that Dr. Belding showed (wherein there was apparent parallelism between the results of the Wassermann test and those of the modified precipitation tests) seems to indicate that all those tests detect the same substance in syphilitic serum or the same modified condition of the serum that is responsible for these reactions. I believe there is no question at this time that complement fixation and precipitation tests, in spite of variability in their technique, detect the same element or elements in syphilitic serum.

THE ABSENCE OF ANTIGENIC ACTIVITY IN THE COMPLEMENT FIXATION TEST OF THE PURIFIED LIPOIDS, CEPHALIN AND LECITHIN. Elizabeth Maltaner (by invitation), Albany, N. Y.

*Abstract.* A study of the antigenic activity in the complement fixation test of samples of purified cephalin, free from lecithin, and purified lecithin, free from cephalin, was made. These preparations possessed no antigenic properties either singly or in mixtures, one with the other. Fortification with cholesterol had no effect. The lecithin was lytic in large amounts, but not anticomplementary. The cephalin was not lytic except in relatively large amounts, but was

*Discussion*

(Dr. Marcus W. Lyon, South Bend.) This phenomenon seems to be correlated with a clinical phenomenon in diseases of the skin. A diseased area of the skin which has been irritated by one substance at one time, subsequently is much more susceptible to toxic substances other than the specific one. That observation has been known to clinicians for a great many years, and a few days before I left I had a clinician call my attention to that interesting phenomenon.

(Dr. Charles Weiss, St. Louis.) I should like to ask if, in the course of examination of the corneas of animals during which you recorded keratitis, you at any time observed pannus.

(Dr. Seegal, closing.) We did very rarely observe pannus.

LOCAL ORGAN HYPERSENSITIVENESS. THE PRODUCTION OF ACTIVE HYPERSENSITIVENESS IN THE RABBIT EYE BY *STREPTOCOCCUS SCARLATINAE* AND ITS PRODUCTS. David Seegal and Beatrice Carrier Seegal (by invitation), New York City.

*Abstract.* It was found that *Streptococcus scarlatinae* filtrate when injected into the rabbit's anterior chamber conferred a specific hypersensitivity in that organ so that the parenteral injection of only the *Streptococcus scarlatinae* or a *Streptococcus erysipalis* filtrate was able to reactivate the sensitized eye. The typical eye reaction could be elicited not only by the parenteral injection of the *Streptococcus scarlatinae* filtrate, but also by the same *Streptococcus scarlatinae* organism living and growing in the subcutaneous tissue of the rabbit. This was accomplished by producing a nidus of infection in the form of an inoculated agar gelatine plaque first described by Dochez. The eye reaction appeared in one to three hours after the injection of the mixture. Plaques containing heat-killed organisms produced no eye reactions. It was further found that the typical eye reaction could be prevented if neutralizing amounts of *Streptococcus scarlatinae* antiserum were injected prior to the administration of the shocking dose of filtrate. A filtrate of a non-scarlatinal *Streptococcus hemolyticus* and a *Staphylococcus aureus* filtrate were unable to relight these eyes.

These experiments seemed to offer evidence that the filtrate of *Streptococcus scarlatinae* could confer a specific sensitivity in the rabbit eye, that the shocking qualities of the homologous filtrate could be elaborated in the distant tissues of the animal and that this agent could be specifically neutralized.

Although the eyes sensitized to the *Streptococcus scarlatinae* filtrate could not be relit by the parenteral administration of a large number of agents, such as egg white, guinea pig red cells and nucleoprotein fractions of the *Streptococcus scarlatinae* bacterial body, it was repeatedly found that the *Streptococcus scarlatinae* filtrate was able to produce the typical eye reaction in rabbits in whose anterior chamber such substances as beef broth, *Streptococcus scarlatinae* bacterial body, nucleoproteins, egg white and glycerine had been injected. These results immediately implied that although *Streptococcus scarlatinae* filtrate could confer a specific hypersensitivity in the rabbit anterior chamber, it also possessed a remarkable ability to cause a non-specific relighting of eyes injected with a large number of heterologous substances. It was at first considered that a simple injury phenomenon originally induced in the eye was the cause of the subsequent relighting process. This might have explained the ability of the eye injected with glycerine to respond to the intravenous injection of the *Streptococcus scarlatinae*

ceived 0.15 cc. of glycerine into the anterior chamber of each right eye under cocaine anesthesia. The glycerine, which contained no protein by the Biuret test, produced a violent sterile inflammation in the eyes, lasting several days. Three intravenous injections of 2 cc. of a 20 per cent solution of fresh egg white were given to each of the animals of this group on the same day as the glycerine injection of the eyes and on the two succeeding days. Animals of Group II received only glycerine into their anterior chambers, but no egg white intravenously, while the third group of animals received only the three injections of egg white intravenously, but no glycerine into the eyes. Three weeks later, when all eyes had returned to a normal appearance, the animals of these three groups received 1 or 2 cc. of a 20 per cent solution of fresh egg white intravenously. In one to two hours the injected eyes of Group I, which had been prepared by intravenous injections of egg white associated with an injection of glycerine into the eye, responded with marked hyperemia of the iris and conjunctiva, lacrimation and edema of the lower conjunctival sac. The animals which received glycerine only into the anterior chamber and those animals which received only egg white intravenously did not respond to this shocking injection of egg white. The accompanying table summarizes these experiments.

	GROUP I Glycerine in right eye and egg white intravenously	GROUP II Glycerine in right eye	GROUP III Egg white intravenously
Three weeks later. Egg white intrave- nously.	Right eye: hyperemia of conjunctiva and iris; lacrimation; edema.	No response	No response

The sensitization produced by this indirect method of injury to the eye associated with intravenous injection of the antigen was similar to the sensitization which we have previously reported following direct injection of the antigen into the anterior chamber. The reaction occurring after the intravenous shocking injection of egg-white appeared in the same time interval of one to two hours, lasted for a similar period of about twenty-four hours, and proved to be specific for egg white. The reaction could be repeatedly elicited by the properly spaced injections of the antigen.

The occurrence of a simultaneous hyperemia in the opposite uninjected eye during the reaction following the shocking injection of antigen was noted frequently in this series of animals. This reaction was at most about half as intense as that in the prepared eye. Woods and Riehm have each reported somewhat similar observations in their studies in sympathetic ophthalmia. They sensitized one eye by direct injection of the antigen and found that later parenteral injection of the antigen produced a bilateral reaction. We have occasionally seen a slight reaction in the contralateral eye in our previous series of experiments, but not as marked as in this group of animals. As yet, we are unable to interpret the reactions in the contralateral eye.

In conclusion, therefore, the observation that an inflamed area of the body becomes more richly saturated with a circulating foreign protein than does neighboring normal tissue has been utilized to produce specific active local sensitization of the rabbit eye.



Some years ago, following a rather extensive sinus operation, I worked a good deal with dried spinach leaves and in the course of about three weeks I had rather violent hay fever attacks every time I came in contact with the leaves. Before that time I had never had any allergic phenomena. At that time no skin reaction could be elicited by a saline suspension of the leaves, which on inhalation would immediately elicit violent attacks. Anyone who has done skin tests on patients with undoubted allergic phenomena has found probably a considerable percentage of patients who will not give the expected skin reaction to the injections, but who on spontaneous administration, as it were, will regularly have an attack. The experiments which were reported, I think, give a very convincing explanation for such a fact, namely that the general assumption is probably not correct that in allergic disease the whole body is prepared to react. It may be only a localized area or one special organ which is sensitized to react with allergic manifestations as soon as the specific allergen reaches the sensitized tissue in sufficient concentration.

PHENOMENON OF LOCAL SKIN-REACTIVITY TO BACTERIAL FILTRATES: PASSIVE IMMUNITY TO REACTING FACTORS. Gregory Shwartzman, New York City.

*Abstract.* It is possible to elicit passive immunity to *B. typhosus* reacting factors by means of normal and immune homologous neutralizing bodies. The "in vivo" serum protection against these factors follows the law of multiple proportions.

Passive immunity is best obtained when the immune serum is injected intravenously one-half hour before the intravenous injection of the reacting factors.

It is also possible to prevent the occurrence of the local skin reaction by an intravenous injection of serum after the intravenous injection of the reacting factors, provided that the serum dose is very large and the serum injection is made immediately after the filtrate injection.

The results also demonstrate passive serum protection against the lethal effect of *B. typhosus* "agar washings" filtrates, suggestively, in multiple proportions.

### Discussion

(Dr. Augustus B. Wadsworth, Albany.) Dr. Shwartzman has made such an important contribution that he deserves to be congratulated. For the past year or two we have followed his work very closely, particularly with antimeningococcus serum. His methods I am sure will be of considerable value as a check or control on the activity of sera used in the treatment of infections, the inciting organisms of which do not produce a sufficiently potent toxin or lack virulence to kill animals, so that a protection test can be used. It is an important method and should be given a thorough trial. I am particularly interested in the mechanism of the lesion, and I wanted to ask Dr. Shwartzman if he would explain his conception of the mechanism of the pathological process in the cutaneous lesion. I have wondered if the process giving rise to the varying degrees of reaction might not be thrombosis leading to the reaction of hemorrhage, so that the extent of the thrombosis rather than the direct action of the toxin might account for some of the variation in the local lesion. I have not had a chance to study many of the sections, and knowing that Dr. Shwartzman has, I should be interested in his interpretation of the pathogenesis of the local lesion.

filtrate, for the glycerine had no protein in it by the Biuret test. However, eyes injured with saline, iodine and albolene failed to respond to the subsequent injection of the *Streptococcus scarlatinae* filtrate.

It was apparent that the agent in the *Streptococcus scarlatinae* filtrate effective in causing the non-specific relighting of the eyes must in all probability be the toxallergen, since this reaction could be specifically neutralized by the *Streptococcus scarlatinae* antiserum. Furthermore, this ability of the *Streptococcus scarlatinae* filtrate to reactive eyes in which these heterologous substances had previously been injected did not appear to be common to several other filtrates studied by us. For instance, a non-scarlatinal *Streptococcus hemolyticus* filtrate and a *Staphylococcus aureus* filtrate were ineffective in producing the eye reactions in these animals. Other filtrates, however, must be tried before conclusions can be drawn on this point. The crucial point is the fact that whereas the *Streptococcus scarlatinae* filtrate is able to induce in the rabbit eye such a specific hypersensitiveness that only the homologous or the *Streptococcus erysipelas* filtrate will incite the characteristic eye reaction; this same filtrate when injected intravenously is able to relight eyes in whose anterior chamber such varied substances as glycerine, broth, egg white and guinea pig red cells have been introduced weeks or months previously.

### Discussion

(Dr. Augustus B. Wadsworth, Albany.) I am very much interested in these reactions and their specific character. The statement that the "scarlet fever" and "erysipelas" organisms were the only ones that reacted specifically, and that the antiserum neutralized this action, is of interest to me. I think it is important, however, to emphasize the fact that any distinction between the streptococci from scarlet fever used in such experiments, and those from erysipelas, depends greatly upon the selection of the strains used. Some of the strains of erysipelas streptococci (I speak of them as "erysipelas" streptococci, not because it is possible to distinguish any particular group of streptococci associated with any particular pathological process by any known means, but simply because the organisms are isolated from these conditions) correspond in their toxic properties with some of the streptococci from scarlet fever. Others do not, and the experiments should be very fully controlled with different streptococcic toxins. The strains from scarlet fever vary in their toxin production. The New York 5 is perhaps one of the broadest strains. When its serum was tested with the toxins of 500 different cultures, between 70 and 80 per cent corresponded. Some of the streptococcus toxins of cultures from erysipelas and from scarlet fever are not neutralized by the antitoxin of the New York 5, whereas others are; also the valency of the toxin as determined by the toxin-antitoxin neutralization with homologous sera varies enormously. In our experience we have only met one or two strains of the broad valency and antigenic action of the New York 5. I think one must control the specificity of the reaction in all such experiments by the accurate determination of the homologous and heterologous action of representative sera in each instance.

(Dr. Max Pinner, Tucson.) The experiments reported may go a long way to explain the very frequent failure to elicit a skin reaction with a specific allergen which apparently brings about the allergic manifestations of disease. I might quote a personal experience which is probably a good example in question.

different preparations, and there were one or two in which there was no loss of potency.

(Dr. Stuart Mudd, Philadelphia.) May I call attention to another phase of this very important procedure on which Dr. Reiner has already published a preliminary paper, namely the possibility of making "charmed bullets" by coupling a substance toxic for a bacterium to the antibody, which will unite the toxic substance with its "target." I suggest that this possibility be kept in mind in the future development of this type of work.

#### STUDIES ON THE PROPERTIES OF BOUILLON FILTRATES OF THE MENINGOCOCCUS.

N. S. Ferry, Detroit, J. F. Norton, Detroit, and A. H. Steele (by invitation), Northville, Mich.

*Abstract.* The meningococcus, when cultivated in hormone broth, exhibits within a few days a luxuriant growth with a peculiar heavy pellicle, and produces in the broth filtrate a toxic substance with properties characteristic of a soluble toxin.

Filtrates from bouillon cultures of the four recognized types of the organism contain toxins apparently type specific, although there appears to be a toxin present in the filtrates common to all types. These filtrates, in high dilutions, produce local skin reactions in a certain number of individuals, depending upon the original strength of the toxin, and whether it is modified by heat or age.

The toxins of the four types, either individually or mixed, when injected into animals, stimulate the formation of antitoxins and these in turn neutralize the homologous toxins in fairly high dilutions. The toxins are also neutralized by meningococcus meningitis convalescent sera and the action is apparently specific to a certain degree.

Lytic extracts of the meningococcus do not act in a manner similar to the bouillon filtrates, supporting the view that the toxins in the filtrates are extracellular in nature.

It is concluded that the various types of the meningococcus produce extracellular toxins in bouillon cultures and that these in turn can stimulate the development of specific antitoxins.

#### *Discussion*

(Dr. Gregory Schwartzman, New York City.) This paper is very interesting to me especially, because of some similarities between the human skin test which the authors reported and the work which I have done on the phenomenon of local skin reactivity to meningococcus filtrates. The first point is the fact that Type 3 meningococcus yields filtrates of higher potency than Type 1. In my work I find that Type 3 meningococcus produces filtrates from two to five times stronger than Type 1. The second point of similarity is the fact that autolyzed bacteria contain practically no toxic substances for either test. I should like to ask Dr. Ferry whether he observed any swelling at the site of the injection similar to that observed in rabbits by Dr. Ecker.

(Dr. Ferry, closing.) As far as I have been able to determine, the toxin from Type 3 in general is no stronger than the toxin from the other three types. This individual Type 3 culture is a rather recently isolated strain and produces a toxin much stronger than any other Type 3 strain which has been growing much longer under artificial culture.

In regard to the question of this filtrate and the reaction which Dr. Schwartzman is getting with his agar washings, I feel compelled to refer to the work

(Dr. Shwartzman.) I shall have to answer Dr. Wadsworth's question by saying that we have not as yet made a sufficient histological study of the lesions. The most obscure thing about the phenomenon is that the second injection has to be given intravenously. One can reinject the same area of the skin with as much as 100 times the amount necessary and not get any reaction. Nevertheless a very minute amount injected intravenously may produce a severe necrosis. I was able to obtain a reaction with a dilution of the meningococcus filtrate as high as 1:2500. I understand that Drs. Wadsworth and Sickles obtained reactions with still higher dilutions. If you consider the fact that this small amount is still further diluted in the blood stream about 100 times and that some of it will be lost in the organs before reaching the prepared skin area, the amount which will actually produce the lesion must be amazingly small. I believe that the fact that the filtrate is only active when introduced through the intravenous channel is intimately related to the mechanism and pathogenesis of this phenomenon. I am not prepared to say just where the first injury in the prepared area takes place, but I am under the impression that the thrombosis is secondary, and that the primary effect is on the endothelial lining of the blood vessels. Dr. Klemperer is conducting some investigations on this point.

(Dr. Ernest A. Pribram, Chicago.) I wish to say that a high dilution of an antitoxin acts in a more delayed fashion than a lower dilution, and this phenomenon has also been observed in diphtheria antitoxin by Hamburger. He also recommended using diphtheria antitoxin in a diluted form. It was an improvement in technique to dilute the serum first and inject it in the diluted form. The sera which are on the market at the present time are usually diluted after the removal of albumin.

MODIFICATION OF THERAPEUTIC SERA WITH A VIEW TO AVOIDING COMPLICATIONS OF ALLERGIC NATURE. J. Bronfenbrenner and (by invitation) Donald M. Hetler and I. O. Eagle, St. Louis, Mo.

*Abstract.* Chemical modification of therapeutic sera according to methods initiated by Obermeier and Pick and by Landsteiner has yielded products which retain a considerable proportion of original antibodies, while they have lost the species specificity of the carrier serum. These products can be administered to animals highly sensitive to horse protein without causing any untoward symptoms. With respect to their antigenic properties many of these preparations are mutually heterologous, thus permitting their use one after another without allergic manifestations.

*Discussion*

(H. M. Powell, Indianapolis, by invitation.) I believe that this line of work is of importance to commercial producers of antitoxin; it appeals to the imagination, and would seem to offer possibilities of a whole series of serums which might be arranged in such a fashion that no one individual would ever need to receive two of the same kind of diazotized serums, and it would obviate the possibility of individuals receiving antiserum which was not completely de-horsed.

(Dr. Augustus B. Wadsworth, Albany.) I did not quite understand the percentage of loss of potency after treatment.

(Dr. R. S. Muckenfuss, St. Louis.) I am not familiar with the figures as I am presenting the paper for Dr. Bronfenbrenner, but I understand it did vary with

do not feel that we have accomplished it, so we are continuing the experiments with different tissues. We have not yet been able to do the experiments you suggest.

**FURTHER OBSERVATIONS ON A SKIN TEST FOR SUSCEPTIBILITY TO POLIOMYELITIS.** Edward C. Rosenow, Rochester, Minn.

*Abstract.* The report consists of a brief presentation of the method used in preparing suitable antigens from the streptococcus isolated, of results of skin tests in persons at the onset of attacks of poliomyelitis, during convalescence and after recovery, and in well persons of different age groups who have not had poliomyelitis, and of the neutralizing power of the serum of "skin test positive" and "skin test negative" individuals.

**THE IMMUNIZATION OF MONKEYS AGAINST THE VIRUS OF POLIOMYELITIS.** William B. Brebner (by invitation), St. Louis, Mo.

*Abstract.* This report deals briefly with experiments showing that many infective doses of poliomyelitis virus may be injected into the spleen of normal monkeys with little risk of the host developing any symptoms of the disease. A short time after the splenic injections it may be shown by neutralization tests that the animals have acquired an appreciable degree of immunity. The significance of these observations as concerns the virus disease group as a whole is considered and mention is made of the importance of "placing" a virus antigen.

### *Discussion*

(Dr. E. C. Rosenow, Rochester, Minn.) I am not quite certain regarding the method of injection of the virus. I understood you to say that both injections were made into the spleen.

(Dr. Brebner.) In all cases the virus was given directly into the spleen.

(Dr. Rosenow.) Was there a rise in temperature following the first injection or following the second injection?

(Dr. Brebner, closing.) The second time that the virus was injected intrasplenically there was a higher temperature of a plateau type, but no symptoms of paralysis. This reaction, in Jungeblut's opinion, is evidence of a sensitization.

**SOME IMMUNOLOGICAL ASPECTS OF LEUKEMIA.** W. C. Hueper and (by invitation) M. Russell, Philadelphia, Pa.

*Abstract.* The high increase of leucocytes in the blood of leukemic patients is not caused by a decrease or absence of the normal antiproliferative action of blood serum upon leucocytes, because it was found that in tissue cultures of normal leucocytes in leukemic plasma this quality was rather enhanced than decreased, if compared with that of normal plasma.

Antileucocytic serum produced by repeated intravenous injection of leukemic leucocytes into rabbits is non-specific in its action against leucocytes, as also the growth of normal leucocytes is completely inhibited by the use of antileucocytic plasma in tissue cultures. The antiserum does not contain any leucolysins. The antiproliferative titer of such sera to be used for therapeutic purposes in leukemias can be determined by the method of leucocytic tissue cultures.

which I carried out several years previously on agar washings, with my associate, Dr. Fisher. The original agar washings were prepared exactly as Dr. Schwartzman's filtrates and at that time we did not consider them very toxic, although they were used for diagnostic skin reactions. As far as I can judge, the toxic material from the bouillon filtrates is entirely different from anything found in the agar washings. The bouillon filtrate contains a soluble toxin, and I feel pretty sure that the agar washings do not. A specific antigen is present in the washings and it may be toxic, but I cannot consider it as a soluble toxin.

In regard to the reaction at the site of injections in the human, as mentioned by Dr. Schwartzman, we have not noticed any swellings similar to those described in rabbits by Dr. Ecker.

#### A STUDY OF ANTIPNEUMOCOCCUS PREPARATIONS BEFORE AND AFTER DRYING.

K. George Falk, Eugenia Valentine, Grace McGuire, and Elinor Whitney  
(by invitation), New York City.

*Abstract.* A method of drying antipneumococcus serum *in vacuo* was described whereby the antibody property was retained intact and could be redissolved. After storage in the icebox for six to twelve months the powdered antisera were the same, or only slightly lower than the homologous untreated sera in their mouse protective units, agglutinin and precipitin titers and in the amount of precipitate with soluble specific substance (Type 1).

When stored at room temperature, on the other hand, the agglutinin and precipitin content had almost completely disappeared and no precipitate was formed with a 1:10,000 dilution of soluble specific substance (Heidelberger), but the mouse protective units had fallen only one-half at the most.

#### NEUTRALIZATION OR DESTRUCTION OF DIPHTHERIA TOXIN BY TISSUE. Augustus B. Wadsworth and (by invitation), Ella N. Hoppe, Albany, N. Y.

*Abstract.* The action on diphtheria toxin of the living animal tissues was studied by using tissue culture methods *in vitro*, and supplementing those with *in vivo* inoculations, for the quantitative determinations. Guinea pigs, guinea pig tissues, and media entirely of guinea pig origin were used throughout the work. Diphtheria toxin was not altered by contact with fetal or adult cardiac tissue in a state of survival. It lost its toxicity if the tissue was growing in a state of cultivation. The tissues were apparently uninjured by the toxin in the dilutions used, and continued to grow uninterruptedly.

#### Discussion

(Dr. Bezi, Rochester, N. Y.) What dose of diphtheria toxin was neutralized by guinea pig embryo cardiac tissue?

(Dr. Wadsworth.) 1:500 MLD in 0.1 cc.

(Dr. N. Popoff, Rochester, N. Y.) Did you try as a control the effect of the monocytes, and an extract of the erythrocytes on the toxin? I ask this question in connection with some work done on the detoxicating influence of an extract of red cells upon toxins.

(Dr. Wadsworth, closing.) In the early days we used only leucocytes, and we have tried from time to time to get a differentiation of these cells—the monocytes. The experiments in the last year have been directed particularly to an attempt to sustain these cells in an active growing state free from other cells, but we

4. The following differences have been established between aureus and albus:
  - (a) Aureus is virulent for rabbits, albus is not.
  - (b) The two strains produce strain-specific agglutinins, which are specifically absorbed only by the homologous strain.
  - (c) Aureus is an active proteolyzer, albus is not.
  - (d) Aureus coagulates citrated blood plasma, albus does not.
  - (e) Aureus produces a hemolysin, albus does not.
  - (f) Aureus produces a higher degree of alkalinity in beef broth infusion than does albus.

5. Rabbits can be rendered completely immune against aureus by injecting them with living albus.

6. Whereas pigment production, virulence and agglutinability go strictly parallel, and are, therefore, dependent on environmental conditions, it was found that the susceptibility to phage remains the same for each strain regardless of its dissociative changes.

7. It is of interest to point out that in staphylococci the phenomenon of dissociation does not involve the usual change in colonial morphology — smooth and rough — but an alteration of pigment production.

#### BLOOD CHEMICAL CHANGES IN EXPERIMENTAL STREPTOCOCCUS SEPTICEMIA.

Richard W. Linton (by invitation), New York City.

*Abstract.* When rabbits are given fatal doses of *Streptococcus hemolyticus* intravenously it is found that some die with fulminating infections within thirty-six to seventy-two hours, and others, which are designated as having acute infections, survive on the average six days.

The following changes have been found to occur in the blood of these animals:

The blood sugar concentration drops at a constant rate throughout the disease, but does not reach a condition of hypoglycemia.

Glycogen is present in the liver at death.

The CO<sub>2</sub> capacity is lowered markedly at first, then returns to a somewhat higher although still subnormal level, at which it continues until the terminal stages of the disease, when the acidosis becomes very marked.

Inorganic phosphorus is markedly increased in concentration at the terminus of the disease. This increase is greater in animals showing an acute course than in those in which the disease is of the fulminating type.

Calcium also shows terminal changes, decreases occurring in the animals with fulminating infections to a greater degree than in those with acute infections. In both, abnormally low levels are reached.

Non-protein nitrogen and creatinine are greatly increased in the terminal stages, in both groups of animals.

It is considered that the results obtained may be explicable on the assumption of a large amount of acid production *in vivo* by the streptococcus. The resulting acidosis becomes uncompensated in the terminal stages of the infection, thus leading to severe changes in the tissues. In the kidney these take the form of an acute diffuse nephritis, which is sufficient in degree to bring about the death of the animal.

#### SPONTANEOUS INSULIN RESISTANCE IN RABBITS: THE EFFECT OF THYROIDECTOMY ON THE RESPONSE TO INSULIN. Isolde T. Zeckwer, Philadelphia, Pa.

*Abstract.* Two rabbits were found showing spontaneous resistance to enormous doses of insulin. One failed to develop convulsions with doses of insulin up to

THE EFFECT OF SUPRARENAL CORTICAL EXTRACT ON RESISTANCE. David Perla and J. Marmorston-Gottesman, New York City.

*Abstract.* Extract of the cortex of suprarenal gland prepared according to the method of F. A. Hartman is a highly potent extract. It is free of toxicity and epinephrin. Injections of cortin will raise the resistance of suprarenalectomized rats to several lethal doses of typhoid vaccine and to several lethal doses of histamine or ergamine acid phosphate for suprarenalectomized rats. The protective action of cortical extract against poisoning with typhoid or histamine in suprarenalectomized rats may be utilized as a means of biologically assaying the potency of such extracts. The amount of cortical extract injected intraperitoneally into suprarenalectomized albino rats on the fifth and sixth day after operation necessary to protect these rats against 200 mg. of ergamine acid phosphate per Kg. of body weight may be considered a standard unit.

STUDIES ON AN INSTITUTIONAL OUTBREAK OF POLIOMYELITIS APPARENTLY DUE TO MILK. Edward C. Rosenow, Rochester, Minn.

*Abstract.* The report consists of a brief statement of methods used and results obtained in a study of the cases that occurred; of the streptococcus isolated from throats and spinal fluid of patients; of the throats of the student body; of the milk from the cows at the dairy supplying the milk; of skin tests at the time of the epidemic and six weeks later at the affected college; and of control studies at a college in the same town where no cases occurred.

EXPERIMENTS RELATIVE TO A POSSIBLE BASIS FOR VACCINE THERAPY IN RHEUMATIC FEVER. B. J. Clawson, Minneapolis, Minn.

*Abstract.* Patients having acute rheumatic fever have been shown to be hypersensitive (allergic) to streptococcic protein.

Animals can be made hypersensitive to streptococci so that lesions will result from later injections with much smaller doses than in normal animals.

The cellular reaction in small lesions in the hypersensitive animal is similar to that found in tissues in human rheumatic infections. There is a marked similarity in the hypersensitiveness (allergy) to streptococci in animals made hypersensitive experimentally and in patients having acute rheumatic fever.

Rabbits made hypersensitive to streptococci can be desensitized by intravenous administration of a streptococcic vaccine which does not have to be type specific.

The results of these experiments suggest the use of intravenous vaccine treatment in acute rheumatic fever.

BACTERIAL PLEOMORPHISM: THE DERIVATION OF NON-VIRULENT STAPHYLOCOCCUS ALBUS FROM VIRULENT STAPHYLOCOCCUS AUREUS. Max Pinner and (by invitation) Marie Voldrich, Tucson, Ariz.

*Abstract.* The relation between *Staphylococcus aureus* and *Staphylococcus albus* is essentially the same as that between S and R strains of other bacterial species.

1. Albus splits off spontaneously from pure line strains of aureus.
2. This splitting off can be hastened and increased by culturing aureus in serum containing aureus agglutinins.
3. From albus strains, aureus colonies can be isolated by culturing albus in anti-albus serum.



may combine with the oil and saponify the oil, as substances which lower the surface tension of a colloid concentrate on its surface. So I should like to ask two questions: Is there a difference in the absorption of substances by the oil (in an aqueous medium), if these substances have a different electric charge and if they contain different diffusible substances, such as the phosphates, which may combine with the oil?

(Dr. Mudd, closing.) If one could introduce an electrode into a red cell and measure the total potential difference (thermodynamic potential difference) between the inside and outside of the cell, possibly the red cell might be positive, although I know of no such measurements. The basophilic and acidophilic nature of the substances in the cell depends upon their dissociation state. I am not discussing either of these things, but am discussing the surface properties of the red cell. The surface charge of the red cell relative to its suspending medium (electrokinetic potential difference) is negative. Any readily adsorbable impurities present in the suspending medium may alter the surface properties of the cell, and errors due to such adsorption effects should be carefully guarded against.

#### FIXATION OF BACTERIA AND OF PARTICULATE MATTER AT THE SITE OF INFLAMMATION. Valy Menkin, Boston, Mass.

*Abstract.* India ink or graphite particles injected into an area of inflammation fail to disseminate to the tributary lymph nodes. When injected into a normal peritoneal cavity they rapidly appear in the retrosternal lymph nodes. When injected into an inflamed peritoneal cavity they are fixed *in situ* and fail to reach the regional lymph nodes.

Graphite particles injected in the circulating blood stream enter an inflamed area both as free particles owing to the increased capillary permeability, and also as phagocytosed material within leucocytes.

Bacteria (*B. prodigiosus*) injected into inflamed tissue are fixed at the site of inflammation and fail to disseminate to the regional lymph nodes as readily as when injected into normal tissue.

Bacteria (*B. prodigiosus*) injected at the periphery of an inflamed area do not readily penetrate into the site of inflammation. The experiments furnish evidence, in addition to that already provided in previous work, that fixation of foreign substances by the inflammatory reaction is primarily due to mechanical obstruction caused by a network of fibrin and by thrombosed lymphatics at the site of inflammation. Fixation occurs very early in the development of the inflammatory reaction (shown in some previous experiments to take place as early as thirty minutes after the injection of the inflammatory irritant). The rapid formation of a fibrin network and of thrombi in lymphatics at the site of inflammation circumscribes the irritating substance and thus prevents its passage into the blood stream. This allows of a definite interval of time for the leucocytes to assemble for phagocytosis. The initial fixation of bacteria or of other injurious substances at the site of inflammation thus becomes a protective mechanism and plays a definite rôle in immunity.

Bacteria (*B. prodigiosus* and *B. pyocyaneus*) injected intravenously rapidly enter an inflamed area. It is suggested that localization of bacteria in a *locus minoris resistentiae* may be explained as the result of increased capillary permeability with subsequent accumulation and fixation of bacteria from the blood stream at the point of injury.

66 units, but went into convulsions with 76 units. The second rabbit required 20 units for development of convulsions. With large subconvulsive doses, the blood sugar remained for many hours near the convulsive level, but apparently the release of glycogen from the liver was sufficient to maintain the blood sugar just above the critical level. During this period, there was frequently exophthalmos, indicating increased activity of the sympathetic nervous system.

In view of the work of Bodansky, of Burn and Mark, and of Britton and Myers, showing that thyroidectomy increased the sensitivity of normal animals to insulin, it was determined to ascertain whether the thyroid was responsible for the abnormal resistance of these two rabbits.

Thyroidectomy was performed under aseptic precautions. The animals became increasingly responsive to insulin as days elapsed after operation, although gaining in body weight. By the twentieth postoperative day, both developed convulsions after 5 units.

The gross and microscopic appearance of the excised thyroids showed no hyperplasia or pathological change.

Apparently the thyroid, although anatomically normal, was functionally responsible for increased sensitivity of the sympathetic nervous system which permitted such ready sympathetic discharge of glycogen from the liver that the blood sugar level could be maintained above the convulsive level after enormous doses of insulin.

#### CERTAIN PHYSICO-CHEMICAL PROPERTIES OF BLOOD AND EXUDATIVE CELLS.

Stuart Mudd and (by invitation) Emily B. H. Mudd, Philadelphia, Pa.

*Abstract.* Application of the interfacial technique permits the study of the wetting properties of cells and of their behavior under compressing and stretching forces. Polymorphonuclear leucocytes are cells of relatively low viscosity, the majority of which disintegrate under the tension of an oil-water interface. The large mononuclears are cells of higher viscosity, the majority of which are comparatively little deformed by the interfacial tension. Lymphocytes and various leucemic cells vary within these limits. All leucocytes studied have *hydrophilic* surfaces, and are strongly resistant to wetting by oil.

Erythrocytes have visible surface membranes, which are capable of withstanding tension. The surfaces of erythrocytes are relatively *hydrophobic*, and offer little resistance to wetting by oil.

The interfacial tension relations at the surfaces of the cells in an oil-water boundary have been analyzed.

#### Discussion

(Dr. Ernest A. Pribram, Chicago.) It would be very interesting to study the physico-chemical differences between the red blood corpuscles and the leucocytes which cause their different reaction to the oil. The red blood corpuscles are positively charged, as they contain the highly positively charged iron. The monocytes have a large basophilic, *i. e.*, negatively charged nucleus and a relatively small protoplasm without granules. The granulocytes or polymorphonucleated cells have a relatively small nucleus and a large protoplasm, which contain neutrophilic, *i. e.*, positive and negative or amphoteric granules. Their electronic position is therefore in between the erythrocytes and monocytes. There are further phosphates present in the leucocytes and monocytes, sodium and potassium salts, which may diffuse through the medium to the oil. They

Of course we looked for lesions in the intima, because that is the striking lesion in tularemia in the walls of the granulomatous abscesses, and I believe it is largely a process of edema and cellular infiltration, and not a true proliferative change in the lung at this stage.

A STUDY OF VACCINE VIRUS PNEUMONIA IN RABBITS. R. S. Muckenfuss, H. A. McCordock and (by invitation) S. J. Harter, St. Louis, Mo.

*Abstract.* A strain of the neurovirus of Levaditi, furnished by Dr. Rivers, and carried by testicular passage, has been used in these experiments. The diluted testicular extract was injected into the lungs by passing a needle through the skin and anterior tracheal wall, or by inserting a catheter through the mouth into the trachea. Thirty rabbits, both normal and vaccine immune, have been studied. After inoculation most of the animals appeared to remain well. Temperature changes were so rare as to be of no significance. Frothy fluid flowed from the nose of a few rabbits that died, if they were held downwards.

The virus incites a characteristic pneumonia in normal rabbits which can be recognized as early as forty-eight hours. In typical cases there are grayish brown to dark red patches of gelatinous consolidation in both lungs, though often more marked in one, and usually situated in the lower lobes, posteriorly. The mucous membrane of the trachea and primary bronchi is hyperemic. If a catheter has been used there is no local reaction. After the use of a needle animals show a marked edematous swelling of the loose connective tissue of the neck, and occasionally of the mediastinum. These animals always show an intense inflammation of the tracheal mucosa at the site of injection.

Microscopically, groups of alveoli are filled with coagulated albuminous exudate. In places this acute edema is the only change seen. Some of the alveoli contain large masses of tangled fibrin; others have a few isolated threads mixed with the exudate. The cellular component of the alveolar exudate varies in different animals, and in various parts of the same lung. The earliest and primary cellular response consists of a mobilization of numbers of large mononuclear phagocytes within the alveoli. Mitoses are numerous in these cells. A few polymorphonuclear leucocytes are occasionally present. In places where portions of the alveolar walls are necrotic, and in alveoli about bronchi with necrotic epithelium, polymorphonuclear leucocytes predominate. Cases that showed bacteria in the lung, either on culture or in stained sections, were discarded.

The most characteristic change is found about the blood vessels. This consists of an enormous dilatation of the perivascular lymphatics which often contain fibrin and many cells. The connective tissue of the adventitia is edematous and at times infiltrated with mononuclear and polymorphonuclear cells. In advanced cases the entire wall of large arteries is edematous and the muscle cells of the media pushed apart. Polymorphonuclear leucocytes often infiltrate all coats of such vessels, and in places collect in groups beneath the intima, lifting it away from the wall.

Both the large and small bronchi show localized areas of necrosis in the mucosa. Hordes of polymorphonuclear cells collect in and about the necrotic epithelium and extend through the bronchial wall. In the submucosa the connective tissue is edematous and the blood vessels dilated.

After extensive search cytoplasmic inclusions of the Guarnieri-body type were found in the bronchial epithelium. They are round or ovoid, have the charac-

TULAREMIC ENCEPHALITIS: PATHOLOGY OF ACUTE TULAREMIA WITH ENCEPHALITIS AND COEXISTING ACTIVE TUBERCULOSIS. F. W. Hartman, Detroit, Mich.

*Abstract.* Gross and microscopic findings in the body of a patient who gave the history of being infected while working in a butcher shop, followed by ulceration at the base of the thumb on the right hand, lymphangitis of the arm and axillary adenitis, then several weeks' course in the hospital, which was characterized by fever and semidelirium, are given. Serum agglutination was shown with *B. tularensis* 1:2500. Grossly characteristic areas of necrosis were found throughout the lungs, lymph nodes and spleen. The brain showed areas of necrosis in the corpus callosum and basal nuclei. *B. tularensis* were demonstrated in sections of these tissues. The left kidney showed characteristic ulcerative tuberculosis in the calyces, and the right epididymis showed an old tuberculous abscess. Tubercle bacilli were demonstrated in these latter tissues in abundance.

*Discussion*

(Major George R. Callender, Washington.) I understand there were no lesions found in the meninges.

(Dr. Hartman.) No.

(Major Callender.) There is one case reported in which the history was typical and the autopsy showed lesions in the meninges, but none in the brain. These lesions I believe were due to *B. tularensis*, but as no agglutination was done, it is not a proved case.

TULAREMIC PNEUMONIA. Howard H. Permar and W. W. G. MacLachlan, Pittsburgh, Pa.

*Abstract.* A histopathological study of a case of acutely fatal tularemia showing an acute diffuse tularemic infection of the lung, a tularemic pneumonia, is presented. Death occurred on the seventeenth day. The clinical course was characterized by extreme toxicity. Consolidation developed in the last few days of life. The autopsy is the ninth reported on tularemia, and the fifth showing a diffuse acute pneumonia at autopsy. The lesion is an interstitial and alveolar pneumonia, with a mononuclear cell response. There are typical miliary necrotic nodules in the interstitial tissues about bronchi and vessels, like those seen in liver and spleen in animals, and in very acute human tularemic infections. The alveolar exudate tends to necrose; and because of a peculiar inflammation of the vessels with narrowing and thrombosis, large areas of the lung tissue become necrotic. The finer air passages are involved, like the alveoli, following the interstitial inflammation. The process is not bronchiogenic.

*Discussion*

(Dr. Alfred Plaut, New York City.) Have attempts been made to find the organisms in the sections, and secondly, is the lesion in the blood vessel not merely a proliferation of the intima itself?

(Dr. Permar, closing.) I believe nobody has ever found organisms in the human tissues. It is possible to find them in experimental animals if the experimental infection has been rapidly fatal. We tried the Giemsa and other stains, and could not find them.

the certain indefinite factors which are inherent to the species possessing the infection.

2. The histopathological nature of tuberculous infections in a given species is essentially the same, regardless of the form or origin of the particular strain of *Mycobacterium tuberculosis* responsible for the lesions.

3. The lesions of tuberculosis in the common fowl usually possess anatomical characters which differentiate them from tuberculous lesions in the lower mammals.

4. These observations would indicate that it would be injudicious to designate a given tuberculous infection as being due to organisms of either human, avian or bovine origin in the absence of the results of pathogenicity tests.

### Discussion

(Dr. Esmond R. Long, Chicago.) I should like to ask Dr. Feldman if any correlation was apparent in these experiments between hypersensitiveness in the animals used, and the type of infection caused in them. The reason I ask the question is this: my associate, Dr. Vorwald, has made a somewhat similar study in which he inoculated with the same type of tubercle bacillus a whole series of animals, including the monkey, rat, guinea pig, chicken, dog, cat, rabbit, and turtle. A varying character of the resulting lesion was noted, which in some respects could be correlated with the allergic state. The more allergic the animal the more likely the tubercles are to be diffuse. In allergic animals the tubercle bacilli, as they spread, fall on sensitized soil. The guinea pig becomes profoundly sensitive to tubercle bacilli, and as the lesion spreads, there is a diffuse type of reaction. The rabbit becomes less sensitized and the tubercles are sharply delimited. The anatomical type of lesion is thus correlated with the variation in sensitiveness in the two animals.

(Dr. Feldman.) We have made no studies of that kind.

(Dr. David L. Belding, Boston.) May I ask if there were any observations made on the number of organisms present in the lesions? One of the characteristics of avian tuberculosis is the relatively large number of organisms present in the lesion.

(Dr. Feldman.) We made studies some time ago with the avian form of organism, injecting it into the brains of dogs, and while the organisms may be demonstrated, they were very few in number, but the same organism in chickens is very frequent.

(Dr. Oskar Klotz, Toronto.) Dr. Feldman has presented an interesting study of the comparative tissue reactions arising in different animals, resulting from infection with human, bovine and avian strains of tubercle bacilli. I am interested to know whether Dr. Feldman has had the opportunity of studying the effects of the avian infection in man. We have had the opportunity of examining the tissues of three cases of avian infection in man, in which the avian organisms were isolated and differentiated by Dr. R. M. Price. In two of these cases there was a generalized glandular enlargement, and they were diagnosed clinically as Hodgkin's disease. Histologically, however, the lesions were not those of Hodgkin's disease, but showed all the characteristics demonstrated by Dr. Feldman in the avian lesions in fowl. It is our belief that the individual tissue reactions are related to the quality of the organism and not solely the peculiarity of the host in which the reaction occurred.

teristic staining reactions, are surrounded by a clear zone in the cytoplasm and often indent the nucleus. These were found in only a few animals and then were present in only a few bronchi. Guarnieri bodies were never seen in the alveolar epithelial cell. A great variety of "inclusions" can be found in the degenerating bronchial and alveolar epithelium, some of which are misleading, but typical Guarnieri bodies possessing the characteristics mentioned, although scarce in our material, where found, can be confused with nothing else.

Immune animals show little or no reaction. Small collections of mononuclear cells in the alveoli and slight edema of the adventitia of blood vessels is usually found. Necrosis is minimal and polymorphonuclear leucocytes are scant. Guarnieri bodies were not found in immune animals.

Normal testicular extract was without effect, while heated virus, virus mixed with immune serum, and herpes virus produced slight reaction that was not to be confused with that of the active virus.

### Discussion

(Dr. Ralph D. Lillie, Washington.) In the work that we did on vaccine virus pneumonia, judging from the pictures that Dr. Muckenfuss has shown, in our material we produced less cellular reaction in the lung, and more of the edema and fibrin type, with more necrosis; the perivascular edema was more marked in our material than in the photographs shown, and we also found bodies which we were inclined to interpret as inclusions, more widespread and more frequently. The virus used, however, was a different strain, and I believe that the differences are to be assigned to that difference in virus. From other work in the literature, I concluded that the virus used by Armstrong and myself produced more necrosis than that of Levaditi or than that used in this work.

(Dr. Max Pinner, Tucson.) Is anything known of the late results, and is there any marked amount of destruction of pulmonary tissue?

(Dr. Lillie.) Our own animals all died. The longest survival we had was eight days, and that showed almost total necrosis of lung tissue over widespread areas.

(Dr. Muckenfuss, closing.) We killed our animals in five to seven days; none were kept longer, and a number of the animals did die, but because of the extreme infiltration around the point of inoculation, we were never certain whether it was a death resulting from strangulation or from pneumonia. In some animals the lung destruction was not extensive.

### A COMPARATIVE STUDY OF THE HISTOPATHOLOGY OF EXPERIMENTAL TUBERCULOSIS IN DIFFERENT SPECIES. William H. Feldman, Rochester, Minn.

*Abstract.* Using pure strains of *Mycobacterium tuberculosis* representative of the human, bovine and avian forms of the organism an experimental tuberculous infection was induced by various procedures in chickens, rabbits, guinea pigs and dogs. From the lesions obtained a comparative study was made to determine if the anatomical character of the cellular reaction in the respective species varied with the bacillary type of the bacteria used to incite the lesions, or if the histological character of the lesions in a given species was constant, regardless of the form of *Mycobacterium tuberculosis* present.

1. Variations in the character of the tissue reaction of *Mycobacterium tuberculosis* are not determined by the bacillary type of the organism *per se*, but by

In all animals a cellular reaction at the limbus was noticeable 6 hours after injection. This was extensive and approximately equal in normal and tuberculous guinea pigs, much less marked in the rabbit and still less prominent in the cat. At 24 hours in all animals polymorphonuclear leucocytes had reached the site of bacillary lodgement in the center of the cornea, in enormous numbers in the tuberculous guinea pig, in less but still large numbers in the normal guinea pig, and in small numbers in the rabbit and cat. At 72 hours a massive abscess was present in the center of the cornea in the tuberculous guinea pig, while in the other animals the zone of inflammation was much more restricted. In all cases, however, it was sharply localized, and the overwhelming majority of cells present were polymorphonuclear leucocytes. In all cases these cells had phagocytosed and brought the bacilli together from a considerable spread of corneal tissue, thus effecting a rapid concentration in a small area.

The results thus confirm previous studies showing that the localization of tubercle bacilli in a restricted area after diffuse infection, but before the development of a true epithelioid tubercle, is brought about by polymorphonuclear leucocytes. The extent of cellular outpouring was a direct measure of the susceptibility of the animal, as far as the different species were concerned. Apparently the less effective the native bacillicidal power of an animal, the greater the mass of its leucocytes called into action at the outset of infection. The increased outpouring of polymorphonuclear leucocytes in the tuberculous guinea pig as compared with the normal, on the other hand, indicates that this is a sensitized cell in tuberculosis and the massive reaction in this case may possibly be looked upon as a manifestation of acquired immunity.

The results throw light on, but do not solve, the question of the origin of the epithelioid cell in tuberculosis. Inasmuch as primitive tissue mononuclear phagocytic cells seem to be few in the cornea, the absence of mononuclear phagocytes in the 72 hour tubercle in this organ, in contrast to their concentration at this time in tubercles in organs where such cells are normally abundant, might be taken as evidence that the epithelioid cell of the tubercle is of local tissue origin. On the other hand, mechanical conditions are more favorable for the influx of the active polymorphonuclear leucocyte in the dense collagenic cornea, than for the sluggish large mononuclears of the blood stream, and this fact may explain the predominance of the former in the tubercle produced in the cornea, and leave open the possibility of tubercle formation by large mononuclears of the blood stream in organs where their exudation and travel occur more readily.

THE EFFECT OF SECONDARY INFECTIONS ON EXPERIMENTAL TRACHOMA. P. K. Olitsky and (by invitation) R. E. Knutti and J. R. Tyler, New York City.

*Abstract.* A study was made of the rôle played by secondary infections in experimental granular conjunctivitis induced by inoculation of human trachomatous tissues or of cultures of *Bacterium granulosis*, and particularly in the reproduction of the florid type of trachoma in animals.

The implantation by a suitable technique of ordinary bacteria on the conjunctivae of monkeys already affected with well marked, characteristic *granulosis* lesions gave rise to a condition showing less predominance of the follicular reaction and more of the hyperemic granulopapillary effect, which resembled closely the florid type of human trachoma. The organisms used — diphtheroids, staphylococci, xerosis bacilli, chromogenic Gram-negative bacilli, spore-bearing Gram-negative bacilli, and non-hemolytic streptococci — were

(Dr. Feldman.) We have had no experience with cases diagnosed clinically as avian tuberculosis. We did some work with Hodgkin's disease, and we have one case which looks as though it might be associated with avian tuberculosis. There was no opportunity to study the lesion such as you mentioned.

(Dr. Stuart Mudd, Philadelphia.) Do all of these lesions progress to death, or do some of them regress? Can you fit into your interpretation tuberculosis of the Yersin type and that which occurs in the white rat, in which the organs are riddled with tubercle bacilli but there is little cellular response?

(Dr. Feldman.) I think it is a fair question to ask about the duration of the lesions. It is reasonable to assume that the character of the lesions might have changed with the duration of time, but in many of the animals, particularly the guinea pigs, the duration of the lesion, especially when the injection was with the human or bovine types, was short. In the chicken the lesions produced by the bovine type extended in one case for 136 days. The bird was perfectly normal in appearance, and when we destroyed it and made sections there were present very definite lesions of tuberculosis, limited, however, to the brain.

(Dr. David Perla, New York City.) It might be interesting in this regard to note that tuberculous infection in the white rat gives a different pathological picture from that in other animals. The tubercle in the white rat, produced by the human, bovine or avian type, consists largely of epithelioid elements. There is no accumulation of lymphocytes or any giant cells of the Langhans type, but just masses of epithelioid cells, and in these one sees tubercle bacilli. After a time giant cells form by fusion of epithelial elements. The nuclei of such giant cells are equally distributed throughout the cell.

(Dr. E. M. Medlar, Mt. McGregor, N. Y.) If you have tubercle bacilli of sufficient virulence in large enough numbers you may not get a tubercle at all. Instead the inflammatory response will go directly to the production of an abscess composed almost entirely of neutrophils without the intervening stage of tubercle formation.

THE CELLULAR REACTION TO INFECTION WITH TUBERCLE BACILLI. EXPERIMENTS ON THE CORNEA IN ANIMALS OF VARYING SUSCEPTIBILITY. Esmond R. Long, and (by invitation) Arthur J. Vorwald and Sion Holley, Chicago, Ill.

*Abstract.* Previous studies by Vorwald have shown that in the lungs of animals of greatly varying susceptibility to tuberculosis the ultimate spread of the disease parallels more often the intensity of the initial response than the reverse; and that in the rabbit prompt phagocytosis of injected tubercle bacilli by polymorphonuclear leucocytes occurs, with subsequent phagocytosis of the latter by large mononuclear exudate cells. The present study was undertaken to study the rôle of the respective exudate cells in the less complex, non-vascular cornea in animals of greatly varying susceptibility. Human type tubercle bacilli in a dosage of approximately 0.001 mg. were injected, under light ether anesthesia, into the center of the cornea in cats and rabbits and normal and tuberculous guinea pigs, previous experience having shown that cats are highly resistant to the strain of bacillus used, while rabbits are less so and guinea pigs are very susceptible. Tuberculous guinea pigs were used, also, to determine the effect of sensitization on the cellular response. Animals of each group were killed after 6, 24 and 72 hours in order to study the character of the immediate reaction.



found, which merely means that this virus is behaving like other viruses. In Boston, Dr. Farber has demonstrated a high incidence, while in the middle west it has not yet been reported, just as the virus causing inclusions in the guinea pig reveals itself by inclusion formation very frequently in some districts of the United States, and not at all in others.

**THE PATHOLOGY AND BACTERIOLOGY OF BRONCHIECTASIS. A STUDY OF EIGHT SURGICAL LOBECTOMIES.** W. L. Robinson and (by invitation) P. H. Greey, Toronto, Ont.

*Abstract.* This represents a study of eight cases of bronchiectasis in which a lobe or portion thereof was removed at operation and sent immediately to the laboratory for culture and sections.

Clinically, most of these represent cases in their developmental stage and therefore offered exceptional opportunity for a study of the incipient stages and the determination of possible etiologic factors. Direct smears, dark field preparations, aerobic and anaerobic cultures of various types, as well as bacterial stains and silver preparations of sections, revealed nothing consistent enough to be pointed to as a specific infective agent.

Cultures were taken from the exudate in the lumen and also from scrapings of the wall. No common flora were recovered. Three cases yielded pure cultures of streptococci of hemolytic, non-hemolytic and viridans types respectively. Two showed mixed aerobic cultures. Two others gave a mixture of hemolytic streptococci, spirochetes, and fusiform bacilli. One other case gave a mixture of pneumococcus Type IV, with spirochetes and fusiform bacilli. In only three of the eight cases, therefore, were spirochetes found.

Histological sections of the bronchi, transverse and longitudinal, showed consistently the same type of chronic inflammatory reaction, namely a lymphocytic and plasma celled infiltration of the whole bronchial wall, most marked in the mucosa. Destruction of the bronchial musculature and elastic sheaths stood out prominently as the dominant factor in the weakening of the wall, leading to the bronchiectatic dilatations. These in some cases were shown to be focal in character, leading to sacular dilatations as well as to the fusiform or cylindrical types usually found. The lining epithelium as a rule consisted of three or four layers of columnar epithelial cells, the surface layer in most cases being ciliated. Under dark field illumination scrapings of these cells showed the cilia whipping for some six or seven hours after removal from the bronchus. In the more acute areas the lining epithelium was quite hyperplastic and irregular in arrangement, and showed occasional small ulcers or erosions with an acute inflammatory reaction in their bases. An endarteritis of the bronchial arteries was also found.

**FOLLICULAR LYMPHOBLASTOMA (GIANT FOLLICULAR HYPERPLASIA OF THE LYMPH NODES AND SPLEEN).** George Baehr, Paul Klemperer and (by invitation) Nathan Rosenthal, New York City.

*Abstract.* Further studies have been made of pathological material obtained from cases reported by Brill, Rosenthal and Baehr as giant follicular hyperplasia of the lymph nodes and spleen in 1925, and by Baehr and Rosenthal before the Association of American Pathologists and Bacteriologists in 1927 as malignant lymph follicle hyperplasia of spleen and lymph nodes. In lymph nodes or spleen obtained at operation early in the disease, enormously enlarged lymphoid follicles characterize the microscopic picture. They resemble huge germinal centers consisting of lymphoblasts, in which mitotic figures are frequently seen.

some of those found in the conjunctiva of monkey and man with different types of granular conjunctivitis. By themselves, they produced only a transient reaction, or none; when inoculated together with material containing *Bacterium granulosis* they did not affect the usual action of this organism, but when introduced into a conjunctiva in which *granulosis* lesions were already well developed, they induced more of the characteristics of the florid stage of trachoma than have been hitherto observed in animals. Notably among the signs were increased hyperemia, edema, and thickening, papillary hypertrophy, obscurity of blood vessels and masking of the follicles. Microscopically these changes were accompanied by increased scar tissue formation, lymphoid and papillary hyperplasia.

THE INCIDENCE OF INTRANUCLEAR AND CYTOPLASMIC INCLUSIONS IN THE SALIVARY GLANDS AND OTHER ORGANS OF INFANTS. Sidney Farber, Boston, Mass.

*Abstract.* A study of the parotid and submaxillary glands of over 200 infants showed intranuclear and cytoplasmic inclusions in the ducts of more than 15 per cent of the cases. These inclusions are of the type originally described as "protozoan-like" structures, and more recently have been associated with certain types of filtrable virus disease. An analysis of the clinical and pathological data reveals no definite disease picture. One case showing these structures in various organs of the body is also described. Photomicrographs illustrating typical findings are presented.

### Discussion

(Dr. Oskar Klotz, Toronto.) I would like to add a word about an interesting finding in a series of rats that was obtained by Dr. J. Thompson in the Nutrition Laboratory of the Hospital for Sick Children, Toronto. These rats, 2 months old, had been subjected to experiments on vitamin D deficiency over a short period. Tissues of the animals were examined histologically and in 70 per cent of them the submaxillary glands were studied. In 14 per cent of these, inclusion bodies were found in these glands which resembled very closely those described by Dr. Farber, as well as those occurring in the guinea pig. These inclusion bodies occupy the nuclei and almost completely obliterate them. There are some differences in size and staining characters between these inclusion bodies of the rat and those of the guinea pig. The animals show no evidence of disease, but microscopically the cells lining the ducts tend to desquamate and sometimes are associated with neighboring lymphoid infiltrations. In some previous work by Cole and Kuttner it was found that the guinea pig virus was not transmitted to rats and it would appear that the inclusion bodies found by Dr. Thompson are specific for this animal. Transmission experiments with the virus from the salivary glands of the rat have as yet not been undertaken.

(Dr. E. V. Cowdry, St. Louis.) I am very much interested in this presentation, because Dr. Wolbach very kindly sent me some of the specimens, and I compared them in detail with those in the guinea pig. I found that it was impossible to distinguish between the two inclusions, although the surrounding tissue is perhaps a little different. I am interested in the incidence of the inclusions, because Dr. Gordon H. Scott of St. Louis attempted to find them in newborn infants and fetuses collected in St. Louis, Minneapolis, and the middle west. He was not successful. In 100 individuals examined no inclusions were

In order to distinguish it from other varieties of lymphosarcoma, the designation "follicular lymphoblastoma" is proposed.

The microscopic pictures shown in the lantern slides illustrate the gradual and progressive change in the pathological process over a period of years from the appearance of giant follicular hyperplasia to a characteristic infiltrating lymphosarcoma.

### Discussion

(Dr. Howard T. Karsner, Cleveland.) I think we have all been very much enlightened by being brought up to date on the question of giant lymph follicle hyperplasia. What impresses me about this presentation, as well as some few personal observations I have made, is the doubt which may arise about the justification of thinking of this lesion as confined to the lymph follicles. Dr. Klemperer, for instance, refers to the increase in the number of follicles in the spleen. I think he would also say, if he used the same terminology, that there is an increase of the follicles in the lymph nodes. Are we justified in referring to the many centra as indication of an increase in the follicles, or as a number of new foci of the disease that are not in themselves primarily lymphfollicular?

(Major George R. Callender, Washington.) One of Dr. Symmer's cases, which was loaned by the Army Medical Museum, terminated with an even more malignant picture than is shown in the slides here. The case was reported in full at the Chicago meeting in 1929, from the Lymphatic Tumor Registry.

ON SO-CALLED HEMATOGENOUS PERITONITIS. Otto Saphir and (by invitation) S. Wile, Chicago, Ill.

*Abstract.* This study is based on seventeen cases of peritonitis in children. The peritonitis was diagnosed clinically as hematogenous in origin. Nine cases were operated upon and the diagnosis apparently confirmed because no primary source of the peritonitis could be detected within the peritoneal cavity. The autopsy revealed in all the cases a primary enteritis and secondary peritonitis. Some of the changes in the intestines were only slight grossly, but histologically were found to be much more advanced. An oxydase stain revealed the presence of polymorphonuclear leucocytes within the muscular coat where they could not be made out by the use of the hematoxylin-eosin stain. A Gram-Weigert stain showed bacteria in the muscularis in some of the cases. In six cases, hemolytic streptococci were cultured from the peritoneal exudate, in two cases pneumococci, and in two cases, *B. dysenteriae* (Shiga), and *B. typhosus* respectively. Cultures taken from the intestinal mucosa revealed hemolytic streptococci in three of these cases, pneumococci in two, and *B. dysenteriae* and *B. typhosus* in two respectively. The peritonitis in these cases is explained by an invasion of the peritoneum by bacteria through the intestinal wall. This type of peritonitis is referred to as *Durchwanderungs-Peritonitis* in the German literature (emigration or migratory peritonitis).

In reviewing the literature on hematogenous peritonitis, difficulties were encountered because many cases are reported without autopsies, or with autopsy but without detailed investigation of the gastro-intestinal tract so as to rule out a migratory peritonitis. The common belief is that a primary upper respiratory infection gives rise to a bacteremia. The bacteremia secondarily produces the peritonitis. There is no explanation why the peritoneum alone should be involved, and no other organ, especially not the bone marrow, which is a favored

The periphery of each large follicle is surrounded by a narrow zone of small lymphocytes with darker staining nuclei. No abnormal cells are found in the blood.

At this stage of the process it can be differentiated from simple follicular hyperplasia of inflammatory or constitutional nature by the frequency of mitotic figures, the greater size of the follicles, the tendency to involvement of the fibrous capsule, and, when the spleen is involved, by the splenomegaly which may reach the weight of 1800 grams. The differential diagnosis from a focal infiltration of the node by Hodgkin's lymphogranuloma can usually be made by the different architecture of the node. The gross appearance of the lymph node is of value, for the huge follicles are often macroscopically visible. The gross appearance of the spleen is also quite different from the porphyry appearance of Hodgkin's disease. In its early phase the condition is pathologically related to the systemic hyperplasias of the lymphatic tissue, although it differs from the common histological picture of an aleucemic lymphadenosis because of the striking follicle formation, and the predominance of lymphoblasts.

In material obtained later in the disease from the same individuals and from four autopsies, the pathological process was seen to have undergone a change. The growth of the pathological follicles resulted in conglomerate fusion so that the characteristic picture of lymphosarcoma had resulted. At this stage, no other diagnosis than lymphosarcoma is possible, the lymph nodes being diffusely infiltrated by polymorphous lymphoblasts with large irregular nuclei, often showing mitoses. The capsule of the lymph node is infiltrated and there is diffuse invasion of the adjacent tissue. But even at this late stage a careful survey of the architecture of the neoplasm still reveals remnants of the original structure. Narrow bands of small dark staining lymphocytes which originally encircled the follicles can still be made out here and there, and with care one can still distinguish the outlines of some of the overgrown follicles.

In four cases the previous splenomegaly was not present at autopsy, in one a splenectomy having been performed, and in the other three cases the spleen having returned to normal size after roentgen therapy. In the latter, the spleen of one, though of normal size, showed gross nodular infiltration and in all these microscopic examination revealed several enlarged follicles presenting the characteristic change. The disease is therefore a form of lymphosarcoma which deserves to be distinguished as a pathological entity because of its characteristic pathology, its unique pathogenetic evolution and its unusual duration. It may form a connecting link between the systemic hyperplasia of the lymphatic tissue and lymphosarcomatosis, the possible transformation of a "pseudoleucemia" into lymphosarcomatosis having been already mentioned by Kundrat. It is clinically distinguishable from the common type of lymphosarcomatosis by its chronicity (one case being still alive after twelve years), the tendency to early general lymphadenopathy and splenomegaly, the complete absence of anemia or cachexia until the end stages of the disease, and the tendency to lymphatic infiltration in the lachrymal gland resulting in unilateral exophthalmos.

Its most remarkable distinguishing characteristic is its radiosensitivity. According to the experience of our roentgen department, there is no neoplastic disease which responds as promptly to relatively small doses of X-ray or radium therapy. Recurrences in distant parts of the body usually occur after varying intervals until eventually, often after many years, they become less easily influenced by radiotherapy.

marrow were positive, but we explained that as secondary to the bacteremia, and the bacteremia secondary to the peritonitis.

(Dr. I. Davidsohn, Chicago.) Was there any relation between the type of organism and the sex? In a number of cases which I had the opportunity to observe we almost exclusively found pneumococci in girls, and streptococci in boys. Did you notice any infection of the genital tract? In some of the cases which I saw, there was evidence of vaginitis in the girls, as a primary infection.

(Dr. Saphir.) Two cases showed pneumococci in the peritoneal cavity; one was a girl and the other a boy. The vagina, uterus, ovaries and tubes were carefully examined, but we found no evidence of inflammation in any of the sections.

(Major George R. Callender, Washington.) Were the pneumococci typed?

(Dr. Saphir.) Yes, they belonged to pneumococci Type II in one case, and Type IV in the other.

(Dr. Norbert Enzer, Milwaukee.) Was there any evidence of pulmonary infection?

(Dr. Saphir.) Two cases showed bronchopneumonia.

(Dr. Shields Warren, Boston.) During this upper respiratory infection was there a positive blood culture?

(Dr. Saphir, closing.) The blood cultures were done after the patients were admitted to the hospital and clinical signs of peritonitis had developed. Therefore no blood cultures were taken early in the disease when the upper respiratory infection occurred.

#### TEETH AND EPIPHYSES IN DEFICIENCY DISEASES. S. B. Wolbach, Boston, Mass.

Presented by Shields Warren, Boston.

*Abstract.* The consequences of a rachitogenic diet and diets deficient in vitamins A and C upon growth of incisor teeth and long bones of rats and guinea pigs are compared and contrasted.

In teeth a rachitogenic diet affects calcification; a C deficient diet causes cessation of formation of the matrix of dentine, and an A deficient diet operates through atrophy of the enamel-forming epithelium and, through atrophy and heteroplastic proliferation of odontoblasts, produces the most profound changes in the teeth of any of the three deficiencies.

In bones a rachitogenic diet affects calcification of cartilage and bone matrices and suspends the sequences of cartilage cell changes preliminary to vascular ingrowth; the proliferative rate of the epiphyseal cartilage is accelerated. The C deficient diet suspends formation of connective tissue, bone and cartilage matrices, resulting in complete solution of continuity at the epidiaphysial region. An A deficient diet suspends growth and brings about conditions approximating those of the adult epidiaphysial region.

Emphasis is laid upon the importance of the repair phenomena in each instance for the understanding of the pathogenesis of the various disturbances discussed. In repair of the rachitic and scorbutic deficiencies one sees normal sequences taking place at an accelerated rate, and the character of the repair here is largely that affecting intercellular substances. In A deficiency repair depends on the restoration of cells to normal states and hence there is a considerable apparent lag in response to restoration of diet.

#### EARLY LESIONS OF RHEUMATIC ENDOCARDITIS. Timothy Leary, Boston, Mass.

*Abstract.* The material presented consists particularly of early lesions of endocarditis obtained from three cases: one dying as the result of violence while in

site of localization in bacteremia in childhood. Furthermore, it is not at all clear how bacteria reach the peritoneal cavity from the blood stream in the absence of an intermediary lesion. It is conceivable that hematogenous peritonitis might occur, but only with intermediary lesions, such as abscesses in the mesenteric attachment or in organs within the peritoneal cavity, or infected thrombi. The peritonitis in these cases, however, is the result of such metastatic pyemic abscesses or thrombi, but does not directly follow the blood stream infection. So-called hematogenous peritonitis, however, is a peritonitis which supposedly directly follows a blood stream infection. There is no proof that such a peritonitis does exist without other metastatic pyemic phenomena. It seems that many cases reported as hematogenous peritonitis are examples of migratory peritonitis (*Durchwanderungs-Peritonitis*).

### Discussion

(Dr. Henry J. Gerstenberger, by invitation, Cleveland.) A case such as Dr. Saphir described was seen in the Babies' and Children's Hospital. It was an infant 8 weeks old who was in extreme collapse and presented sclerema. A peritoneal puncture revealed a cloudy fluid and a lumbar puncture also presented pathology, as the fluid contained an increased number of cells. I would like to ask Dr. Saphir if in any of the cases reported by him the lumbar fluid was examined.

(Dr. Saphir.) There was no evidence in any of our cases of involvement of the meninges, and a lumbar puncture therefore was not done.

(Dr. Gerstenberger.) I might explain why we did a lumbar puncture in this case. Oftentimes in these infants the general picture does not seem to be that of a peritonitis, but rather that of an encephalitis. As a matter of fact, the first patient with peritonitis that I had occasion to see was diagnosed by me as encephalitis because of the absence of peritoneal signs and the presence of severe cerebral collapse. The lumbar fluid, however, in this first case was negative, and the autopsy revealed the presence of a peritonitis.

(Dr. E. T. Bell, Minneapolis.) I should like to ask if these changes in the wall of the intestine might not be interpreted as secondary to the peritonitis and not as primary. In any case of advanced primary peritonitis you see changes in the muscle of the intestine. It would be curious if a mild enteritis should cause a peritonitis when we know that a frank outspoken enteritis does not cause peritonitis, except in perforation.

(Dr. Saphir.) We have control sections taken in cases of perforation of the intestine. In those sections which were taken from other portions than from the site of perforation, and which were stained for oxidase granules, we could not find any polymorphonuclear leucocytes in the muscularis. In many of the cases the enteritis was grossly much less marked than might have been expected from the histological picture. It does not seem possible to me that a primary peritonitis should produce a secondary enteritis extending over long stretches of the intestine—for instance, over the entire gastro-intestinal tract. It does not seem to me that the enteritis in such cases could be secondary to the peritonitis.

(Dr. David P. Seecof, Cleveland.) I should like to ask if the bone marrow from these cases was examined culturally.

(Dr. Saphir.) The bone marrow was examined microscopically and grossly in some cases, but nothing was found. In a few instances the bone marrow was cultured, and nothing found. In two or three cases, the cultures of the bone

(Dr. E. C. Rosenow, Rochester, Minn.) The lesions in the valve shown by Dr. Leary remind me very strongly of the lesions in the valves which I found in rabbits following the intravenous injection of *Streptococcus viridans*. Ulceration and vegetation formation are usually found first along the line of closure, even though the initial lesion is subendothelial, and in this beautiful example, where the bacteria are numerous, and where there is ulceration, why could not these masses in the depth of the valve be the cause of the thin fibrinous deposit on the surface, which so strikingly seems to be from the circulation? In the experiments in rabbits it was demonstrated that ulceration along the line of closure suggesting implantation endocarditis, was nevertheless secondary to antecedent embolic subendothelial hemorrhage and growth of the streptococcus. This is obviously difficult to demonstrate in the human, but readily accomplished in experimental animals by sacrificing them at intervals following injection. Capillaries surrounding the embolic lesion were not always demonstrable.

(Dr. Leary, closing.) I had in mind, of course, Dr. Rosenow's splendid demonstration of the production of embolic endocarditis when I discussed this case. We could not find any vessels. There are two possibilities: the bacteria were deposited on the surface from the overflowing blood, or they were delivered embolically. No matter how they got there, the action of the closing and opening of the valve is a mechanism adapted for the distribution of the organisms over the contact edge of the valve. We could not find any vessels on careful search which might cause an embolic lesion at the point where the bacterial mass was found.

#### GLOMERULAR LESIONS IN ENDOCARDITIS. E. T. Bell, Minneapolis, Minn.

*Abstract.* The embolic lesions described by Löhlein were for a long time considered the only glomerular alteration in bacterial endocarditis. Baehr and Lande, however, called attention to the frequent occurrence of acute diffuse glomerulonephritis in association with bacterial endocarditis. Clawson and Bell corroborated this observation. In this study I have found that about two-thirds of all cases of bacterial endocarditis and about one-third of all cases of acute rheumatic endocarditis show a definite acute diffuse glomerulonephritis at postmortem.

In preparations stained with hematoxylin-eosin the glomeruli show a definite increase of nuclei, but they are smaller and the capillary obstruction is much less than in clinical acute glomerulonephritis.

Preparations stained by the Mallory-Heidenhain method (McGregor) show a striking increase in the number and size of the capillary endothelial cells. It differs, however, from clinical acute glomerulonephritis in the absence of intracapillary fibers and in the fact that the capillary obstruction is not so pronounced.

A similar increase of glomerular endothelium is frequently seen in persons dying of septic processes such as lobar pneumonia and streptococcic bacteremia.

The presence of large numbers of bacteria in the circulating blood often causes glomerulitis.

#### Discussion

(Dr. Paul Klemperer, New York City.) I should like to ask Dr. Bell in how many cases of subacute bacterial endocarditis he found actual glomerulonephritis of the clinical type. These lesions which Dr. Bell has demonstrated are very often found by us in subacute bacterial endocarditis, I think in about the

apparent full health (Case I); one dying on the way to the hospital (Case II), and one dying of organic disease unrelated to the cardiac process (Case III).

These cases present a valvular lesion marked by the formation of a palisade of cells lining the contact edges of the valves. The early stage of this lesion has not been described. In Case I (early phase) the palisade was covered by a thin layer of fibrinous exudate throughout which Gram-positive diplococci were distributed singly or in small masses. In the necrotic valve edge at one point a larger mass of bacteria was found either lying in a vessel or in a crevice in the valve surface. There were no vessels in its neighborhood. There was no acute inflammatory process in the valve tissues. Cultures developed a pure growth of *Streptococcus viridans*. There were no verrucae on the valve.

Case II (intermediate phase) showed an old mitral stenosis. On the tricuspid valve occurred crops of verrucae and a yellow-brown layer traversing the whole contact edge. The yellow-brown layer consisted of a palisade covering the intervrrucal region and the surfaces of the verrucae. All stages in the evolution of the verruca from early budding processes to advanced stages were present. In addition diffuse formation of scar tissue direct from the palisade cells could be demonstrated. No bacteria were found.

In Case III (later phase) an old degenerating stage of the palisade persisted on a chronic mitral valve on which pseudoverrucae had formed. These were made up of clots deposited on the necrotic scarred valve edge. This stage has been described and illustrated by Königer. Remains of the palisade were observed on rheumatic valves from other cases in the verrucal stage, in which the process was not too advanced.

The lesion represents, apparently, a reaction of allergic tissue to injury. The presence of diplococci on the surface of the valve showing the earliest, most perfect process, is suggestive.

As Poynton has indicated, our difficulties with reference to etiology and diagnosis in rheumatism are "increased by the great rarity of fatal rheumatic heart disease in the very earliest phases of the illness in man." The lesions described in this paper, it is believed, represent an early process whose character helps to elucidate the evolution of the classical picture of verrucose rheumatic endocarditis.

### Discussion

(Dr. Benjamin J. Clawson, Minneapolis.) I should like to ask Dr. Leary if in the course of his coroner's work he has seen an aortic lesion with extreme calcification, the so-called arteriosclerotic valve, and what is his interpretation concerning the etiology and pathogenesis of this aortic lesion?

(Dr. Leary.) I have already discussed this question with Dr. Clawson, and we are in agreement. It is my opinion that this calcified lesion of the aortic valve referred to as arteriosclerotic is basically of rheumatic origin. I think the end result is calcification. I know that this is not in general agreement with the world at large, but there are a good many things on which Dr. Clawson and I agree, and which do not agree with the world at large.

(Dr. Norbert Enzer, Milwaukee.) I should like to ask Dr. Leary if he stained organisms in the valve as well as on the surface of the valve.

(Dr. Leary.) I did not make it plain that the exudate must have arisen wholly from the blood flowing over the surface of the valve. There were no bacteria in the valve other than this mass which I described. We could not find any vessels, and the valve was not vascularized, except at the base.



abscesses are two types of large mononuclear cells. One type is quite definitely the cell called the mononuclear leucocyte, or endothelial leucocyte. The cells of this strain are phagocytes and fuse to form foreign body giant cells. The other kind resembles the type of cell which Dr. Medlar has just described so closely that I am unable to differentiate them. They respond to the monilial reaction as inflammatory cells and then pass to nearby lymph nodes and may occasionally be seen there in mitosis. I have not been able to reproduce the picture of Hodgkin's disease in spite of the fact that typical Hodgkin's cells have occurred in the lesions. It seems to me necessary to assume a tumor-like character to these exudative cells to complete the lesion of Hodgkin's disease. For example, if we assume that certain wandering cells in persons susceptible to Hodgkin's disease may possess tumor qualities, may be capable of responding as inflammatory cells to any one of several bacterial stimulants, and may then pass to the regional lymph nodes and take on accelerated neoplastic growth, we have a theoretical explanation of the disease which coincides with the pathological findings. If no tumor quality is necessary, it seems that some of the irritants which call out the type cells in animals should occasionally produce the picture of Hodgkin's disease, and so far that has not been possible.

(Dr. J. P. Simonds, Chicago.) I should like to ask Dr. Medlar if he has any information in regard to the number of blood platelets present in the circulating blood in Hodgkin's disease. Some work by Dr. F. D. Gunn in our laboratory shows a definite correlation between the number of platelets in the circulating blood and the number of megakaryocytes in the bone marrow. He was fortunate enough to isolate from a subcutaneous abscess in a rabbit a bacillus which has a specific action, in that it stimulates the production of blood platelets up to, in one or two instances, three and a half million per cubic millimeter of blood, and there was a corresponding increase in the number of megakaryocytes in the bone marrow. If the giant cells in Hodgkin's disease are megakaryocytes, it would be interesting to know whether they contributed blood platelets to the circulating blood, just as those in the bone marrow do.

(Dr. Medlar, closing.) Bunting has shown, years ago, that there is a marked increase in the blood platelets, and Minot has found immature megakaryocytes in the circulating blood in Hodgkin's disease. An increase in blood platelets is not specific for any one thing. They are increased in the circulation in many acute inflammatory processes. An increase in blood platelets is often observed in acute tuberculosis. Megakaryocytes are occasionally found in large numbers in the lung in acute lobar pneumonia. It appears to me that the megakaryocyte and the blood platelet play an important part in early acute inflammatory reactions.

UNATTACHED TUMORS FROM THE EMBRYONAL UROGENITAL APPARATUS. G. H. Hansmann and (by invitation) J. W. Budd, Iowa City, Iowa.

*(Abstract not submitted.)*

ON THE PRODUCTION OF DECIDUAL CELLS IN AN OVARY ABOUT A PRIMITIVE CHORIO-EPITHELIOMA OF THAT ORGAN. Charles Simard (by invitation), Montreal, Canada.

*Abstract.* A primitive chorio-epithelioma of the ovary occurred in a woman aged 42, whose last confinement occurred six years ago. Since that time there was no evidence of normal or ectopic pregnancy.

same percentage as Dr. Bell has mentioned. They were especially impressive in cases in which the influenza bacillus or the gonococcus was the cause of the subacute bacterial endocarditis. In addition, however, we find in a certain number of cases actual glomerulonephritis. Furthermore, a type of renal lesion occurs where there is also clinical evidence of renal damage with death in uremia, in which so great a number of glomeruli are destroyed by the focal emboli glomerulitis that the kidney becomes insufficient.

(Dr. Bell, closing.) There are a few of these cases, I do not recall the actual number, in which there was a definite clinical acute glomerulonephritis, but it is difficult to decide that. If there is edema present, it might be due to the decompensated heart, but in a considerable number in which the blood chemistry has been studied, there is a marked nitrogen retention, so that perhaps some of these may be put in the group showing renal insufficiency. I do not think this lesion is due to any specific organism. It occurs with pneumococci, both kinds of streptococci, hemolytic and non-hemolytic, and rarely with staphylococci.

AN INTERPRETATION OF THE NATURE OF HODGKIN'S DISEASE. E. M. Medlar,  
Mount McGregor, N. Y.

*Abstract.* The material made available for this study consisted of tissues from 22 autopsies of Hodgkin's disease and about 100 surgical specimens diagnosed as lymphoblastoma, lymphosarcoma, and Hodgkin's disease.

Evidence was found which made it seem probable that Hodgkin's disease is a dyscrasia of the hematopoietic tissue of the bone marrow. It appears that the "stem" or parent cell of the hematopoietic tissue of the marrow is a multipotential cell which gives rise to all of the cell types of the blood known to be produced in this tissue. This leads one to believe that Hodgkin's disease is closely related, genetically, to the myelogenous leukemias and to the erythroblastic dyscrasias. The essential difference between these dyscrasias is that in neutrophilic myelogenous leukemia the parent cell differentiates mainly along the line of the neutrophile, while in Hodgkin's disease the differentiation is chiefly along the line of the megakaryocyte. The complex pathological manifestations of marrow hematopoiesis, such as seen in pernicious anemia, myelogenous leukemia, and Hodgkin's disease would, from the evidence found, revert to a single type of cell which is multipotential. To emphasize that all of these pathological processes are concerned, genetically, with but a single type of cell, the author suggests that such a term as myelogenous leukemia or Hodgkin's disease should be used, merely to indicate that the main line of differentiation of the parent cell is toward the neutrophile or eosinophile or the megakaryocyte.

The author is of the opinion that Hodgkin's disease is a neoplastic manifestation of bone marrow hematopoiesis and that the involvement of tissues outside of the marrow may be regarded as metastatic tumor growths. To indicate that the multipotential parent cell differentiates mainly along the line of the megakaryocyte and blood-platelet in Hodgkin's disease, the term megakaryoblastoma is suggested.

*Discussion*

(Dr. Samuel R. Haythorn, Pittsburgh.) I have been very much interested in Hodgkin's disease from the standpoint of the cytology, though I am by no means sure of the sources of the so-called "Dorothy Reed" cells. We have been working with a strain of *Monilia* which produces abscesses. In the walls of these

Infections coincident with or consecutive to lacerations and in association with erosions, ectropion, retention cysts and continued irritation constitute frequent composite lesions of importance second only to neoplastic changes.

Precancerous criteria may be interpreted only as possible indications of ultimate cancer development.

The grading of malignant growths should be influenced by clinical data.

### *Discussion*

(Dr. Alfred Plaut, New York City.) I was very much interested in hearing about forty benign tumors of the uterine cervix in 1000 cases. I would like to hear what kind of tumors they were. In a large number of cervixes I have observed, for five and a half years, I have seen a much smaller number of benign tumors.

(Dr. Davis.) There were six myofibromas, one endothelioma, and thirty-seven polyps.

(Dr. Plaut.) I never classify polyps as tumors.

(Dr. James Ewing, New York City.) I should like to ask Dr. Davis if he has any evidence that these carcinomas begin from what he calls the repair epithelium, or from the original epithelium.

(Dr. Davis, closing.) I was greatly impressed in this study by the changes taking place in the repair epithelium, in the presence of long continued inflammatory reaction. The picture of active repair, epithelial proliferation, nuclear polymorphism, and stromal scarring was repeated so many times that it became very impressive in sections definitely malignant and in those without malignancy. I think in a few sections where the repair epithelium was of second degree, that is, the first repair epithelium had been denuded and the second repair epithelium had appeared, that type of epithelium appeared peculiarly sensitive to continued infection, showing an appearance of unstable basal cells.

REPORT OF A SERIES OF ADRENAL ADENOMAS. Charles F. Branch, Boston, Mass.

*Abstract.* A series of seven cases of adrenal adenoma occurring within one year in a series of approximately 300 autopsies is reported. In three cases the tumors were bilateral, the largest measuring 2.5 cm. in diameter, the smallest 1.2 cm. in diameter. All were perfectly encapsulated, discrete masses. Microscopically they consisted uniformly of irregularly arranged groups of cortical cells, having a high fat content. It is interesting to note the complete absence of any clinical significance of the tumors. No two of the cases died from the same cause. The individual ages varied from two in their eighties to the youngest, 41, who died of a recurrent acute rheumatic fever. Four were in females, three in males. On careful examination no endocrine disturbance of any note could be found in even a single case.

### *Discussion*

(Dr. David P. Seecof, Cleveland.) I should like to emphasize, as Dr. Branch pointed out, the association of these so-called adenomas with age. I do not know the exact figures, but in the autopsy series at the Montefiore Hospital in New York it would be safe to estimate that in people over 50 or 60 years of age such adenomas occurred in the adrenal in over 40 per cent of the cases.

The other point is the so-called adenomatous nature of these. I have come to regard the growths in the adrenals just as we regard the adenomas in the

The tumor is formed by strands which recall the chorionic villi of the placenta. They are bordered by large, flattened syncytium masses. Beneath the syncytial layer are cells that are similar to Langhans cells.

The tumor is a typical chorio-epithelioma.

In the ovarian tissue, but outside the invaded zones, are found sheaths of multistratified cells around capillaries or small vessels. Those elements are characteristic decidual cells.

In the case reported, where there are neither follicles nor corpus luteum, we have to deal with a tumor of pure chorioplacental elements, therefore with elements which represent the sole fetal placenta. Then it is not illogical to believe that the appearance of decidual cells is caused by a secretion of the chorio-epithelioma elements.

If the perivascular decidual cells of the tumor have been differentiated under the hormonal action of the chorio-epithelioma, it seems that the hormone which gives rise to the appearance and multiplication of the decidual cells, in normal pregnancy, may come exclusively from chorioplacental elements.

In other words, if the interpretation is correct, it suggests the existence of a hormone which, secreted by the elements of the fetal placenta, determines the production of the characteristic cells of the maternal placenta.

#### GRADING OF CARCINOMA OF THE CERVIX UTERI AS CHECKED AT AUTOPSY. Shields Warren, Boston, Mass.

*Abstract.* While the grading of malignancy is not uniformly applicable to the individual case, it is not infrequently of value, particularly from the standpoint of determining radiosensitivity. The duration of life alone does not give a fair criterion of the malignancy of a given tumor, as many persons do not die of the tumor itself, but of other conditions, which may or may not be related. While perhaps one-sided, the power of metastasis is a useful objective criterion, particularly when the extent of metastases is determined postmortem.

When a given case is followed through to death, variations in the clinical factors do not have to be so heavily weighed; variations in type and efficacy of treatment are not so important; and the grading of the tumor can be based on the entire specimen rather than on a biopsy, which may or may not accurately represent the tumor as a whole.

This study is based on seventy-six autopsied cases of carcinoma of the cervix. A modification of Broder's classification was employed, recognizing the three grades of epidermoid carcinoma, the adeno-acanthoma, and the adenocarcinoma.

In two of the fatal cases no tumor was found at autopsy. Of the twenty-one cases of Grade I epidermoid carcinoma, 86 per cent either did not metastasize, or their metastases were restricted to the regional lymph nodes. Of thirty-four Grade II epidermoid carcinomas, 44 per cent were so restricted. All of the four adeno-acanthomas had spread beyond the regional lymph nodes. Twenty-five per cent of the four adenocarcinomas were restricted to the regional lymph nodes. Thirty-six per cent of all cases examined showed visceral metastases. The commonest single cause of death was obstruction of urinary outflow.

#### PATHOLOGY OF ONE THOUSAND CERVICES. James E. Davis, Detroit, Mich.

*Abstract.* Consecutive examination of a large group of cervixes stimulates new evaluations.

(Dr. Eisenhardt.) In order to have good photographs for demonstration, I spend considerable time in finding the best area possible in the supravital preparations, but I spend an equal amount of time on the fixed preparations of the same tumor in order to select the best areas for comparison. Perhaps you use too much pressure on the material, which tends to destroy the cells. In a pituitary adenoma, for example, the cytoplasm is so fragile that only very gentle pressure should be used. The tissue ought to be examined very promptly also. Possibly your neutral red dye is not satisfactory.

(Dr. Boyd.) Should normal tissue give you the same staining reaction as the tumor does?

(Dr. Eisenhardt.) Yes. Possibly you will recall how clearly the muscle fiber invaded by tumor was seen in the supravital preparation.

(Dr. Esmond R. Long, Chicago.) I should like to mention a simple method, very crude in comparison with the one described, but of some use for quick work and where there are many sections to examine. It is to take a fresh surface and to touch it lightly to a slide, thus making a stamped impression of the tissue. A good many of the cells come off in their original interrelationship. You can see the original parenchymatous cells and the interstitial cells of inflammation, for example, and thus a rough impression is given of what is present in the tissue.

#### THE DIFFERENT TYPES OF CIRRHOSIS OF THE LIVER. F. B. Mallory, Boston, Mass.

*Abstract.* About twelve different types of lesions of the liver may terminate in sclerosis of the organ. Some are acute in character, others chronic and progressive. In the order of frequency they are as follows:

1. Alcoholic cirrhosis: about 50 per cent, is characterized by the presence of an acidophilic reticulum within the affected liver cells. A marked deposit of fat in the cells is a frequent complication, especially in the early stages. The islands of regeneration are usually not over 1 to 3 mm. in diameter. The lesion is not caused by ethyl alcohol, but probably by some toxic substance frequently contaminating liquors.

2. Pigment cirrhosis: about 9 per cent, is characterized by the presence of two yellow pigments, hemofuscin and hemosiderin, in the liver cells, in the bile duct epithelium, in the smooth muscle cells of the blood vessels, in fibroblasts, endothelial cells and macrophages. The islands of regeneration usually measure 1 to 3 mm. in diameter. The new liver cells contain no pigment at first, and they acquire hemofuscin granules which gradually change to hemosiderin granules. Slow necrosis of scattered liver cells is followed by regeneration. The lesion is probably due to copper which combined with hemoglobin is deposited as yellow pigment granules in the liver cells. The copper soon disappears and leaves behind hemofuscin which in time changes to hemosiderin.

3. Acute toxic cirrhosis: about 8 per cent, follows extensive acute necrosis, often involving all the liver cells in many lobules. The necrotic cells are dissolved and removed by the action of leukocytes, leaving large areas of red atrophy containing only sinusoids, stroma, proliferated bile ducts and blood vessels. The islands of regeneration are usually large, often 1 cm. or more in diameter. This type of lesion is due to a variety of toxic substances (phosphorus, arsenic, bacterial toxins).

4. Syphilitic cirrhosis: about 4 per cent, is due to *Treponema pallidum*. The lesion occurs as a diffuse sclerosis, especially in the congenital form, second-

thyroid and prostate. They are not really adenomatous, but localized areas of hyperplasia within misplaced adrenal rests. They occur only at puberty or the prepuberty periods. We would not expect signs of virilism in people 50 or 60 years of age.

#### FURTHER STUDIES OF INTRACRANIAL TUMORS BY SUPRAVITAL TECHNIQUE.

Louise Eisenhardt (by invitation), Boston, Mass.

*Abstract.* An additional series of illustrations selected from the last hundred intracranial tumors which have been studied by this method, upon which the surgical clinic has come to depend more and more for immediate diagnosis, is presented.

#### *Discussion*

(Dr. Max Levine, New York City.) I should like to ask Dr. Eisenhardt if she has compared the supravital preparations with preparations made after fixing with some of the better cytological fixatives, and how long these supravital preparations last? They serve a purpose, but do they last?

(Dr. Eisenhardt.) They have not very lasting qualities. We use the method mainly for the purpose of immediate diagnosis, in order that the surgeon may know what kind of tumor he is dealing with, and what operative method to pursue. As fixatives we use formalin and Zenker solutions, except when special stains are to be prepared.

(Dr. Marcus W. Lyon, Jr., South Bend.) About forty years ago, when I was a student in zoölogy, we used to be able to fix hydroids with all the tentacles extended by pouring hot bichloride solution on them. It seems to me it ought to be possible to fix tissues so that they would retain their natural form just as easily as hydroids and barnacles. I think the trouble is in using poor fixation methods.

(Dr. Eisenhardt.) One of the purposes of using unfixed tissue is to study the cells while they are alive. We keep the preparations in a warm box, and observe the activity of the cells. We have permanent slides made at the same time of all the specimens examined.

(Dr. James Ewing, New York City.) I think the communication of Dr. Eisenhardt is of considerable importance. It shows it is not necessary to wait for the disintegration produced by the ordinary fixing and staining methods in order to get a diagnosis. For a quick diagnosis, these sections seem even superior to frozen sections. At the Memorial Hospital we have very largely abandoned frozen sections, and instead of that method we employ aspiration, putting in an 18 bore needle. One can get from almost any tumor sufficient fragments of tumor which are fixed in the air and stained with hematoxylin and eosin, and have a diagnosis in two or three minutes. That does not give you the advantage of the study of the living cell which Dr. Eisenhardt is particularly interested in, but it gives a diagnosis very quickly, and the cell forms are much better preserved than by other methods.

(Dr. William Boyd, Winnipeg.) After listening to Dr. Eisenhardt last year and looking with great admiration at her pictures, I tried out her method with fresh animal tissue. The result was a complete failure, and I was unable to get any satisfactory differentiation of cells. I should like to know, first, what kind of picture normal fresh tissue gives, and secondly, whether there are any special technical pitfalls into which I may have fallen. I wanted to try the method on normal tissue before using it on tumors.

fall to very low levels, even with a slight injury of the liver. It is believed that bile is essential for normal existence, and animals deprived of it for weeks develop abnormal conditions leading shortly to death. It appears that bile salts are the most essential constituent of the bile and have many important interrelations within the body. Liver feeding may prevent certain types of intoxication in bile fistula dogs.

The second point is that the liver is essential to the construction of blood hemoglobin. Under carefully controlled conditions in anemia, the animal produces uniform amounts of new hemoglobin; when the liver is injured in certain special ways the output of hemoglobin under similar conditions may be decreased to 50 per cent normal. This indicates that the liver takes a part in the building up of hemoglobin in the body. Probably it helps in the assembling of amino acids to form the parent substances of hemoglobin utilized in the bone marrow, where the red cells are finished and turned out into the circulation. This observation is in harmony with the well known fact that the feeding of liver enables an animal or human being to make large amounts of new blood, and therefore explains the use of liver diet in anemia.

A CLINICAL AND PATHOLOGICAL STUDY OF 297 CASES OF CIRRHOSIS OF THE LIVER. James S. McCartney, Jr., Minneapolis, Minn.

*Abstract.* In this series of cases 87 per cent are of the portal or Laennec type, obstructive biliary and syphilitic cirrheses making up the remainder. Of the portal type approximately two-thirds are considered as clinical and one-third as latent. Portal cirrhosis is more frequently latent in males than in females, whereas the clinical cases are about equally divided between the two sexes. The peak of incidence in the latent cases (30 per cent) is in the seventh decade; in the clinical cases it is in the sixth decade (27 per cent). Usually the livers from latent cases show, on microscopic examination, a less advanced process than is shown in the clinical cases, although an advanced cirrhosis is frequently present in the latent group. Small cirrhotic livers are much more frequently found in clinical cases than in the latent group, 25 per cent of the livers in the former group weighing 1000 gm. or less, as against 5 per cent in the latter. Livers weighing 2000 gm. or more are observed in about 25 per cent of each group. Spleens weighing 250 gm. or more are present in 66 per cent of clinical cases and in 39 per cent of latent cases.

EXPERIMENTAL PIGMENT CIRRHOSIS OF THE LIVER DUE TO COPPER POISONING.

E. M. Hall and (by invitation) E. M. MacKay, Los Angeles, Calif.

*Abstract.* Methods. Healthy rabbits were selected. For each rabbit to be fed on copper, a litter-mate of the same sex was selected as a control. Each series when 85 days old was placed on a special diet consisting of a mixture of ground alfalfa and ground barley. When 90 days of age, one series was placed on a diet in which 2 mg. of copper were added to each gram of the special diet. These diets and tap water were allowed *ad libitum*. Many of the copper-fed rabbits died after several weeks, due to copper poisoning, while none of the control rabbits died before being sacrificed.

Results. Seventeen out of twenty-one copper-fed rabbits showed pigmentation of the liver. The pigmentation consisted mainly of hemofuscin granules which were found closely packed in large Kupffer multinuclear giant cells. A few of the livers contained hemosiderin in small amounts. Ten out of twenty-

ary to the action of the organisms on the stroma; and as infarcts (gummas) resulting from occlusion of blood vessels (the usual form of the lesion in acquired syphilis). Organization of infarcts leads to extensive scar formation and retraction (hepar lobatum).

5. Colon bacillus cirrhosis: about 4 per cent, is due to invasion of bile ducts (cholangitis), rarely of portal veins (pylephlebitis), by the colon bacillus. The lesion may be acute, recurrent, or continuous. It is located at the peripheries of the lobules, spreads towards the hepatic veins, and is often associated with obstruction of the common bile duct. Proliferation of small bile ducts is sometimes marked.

6. Obstructive cirrhosis: about 3 per cent, is due to occlusion of bile ducts, usually by calculus or cancer. The lesion is mechanical in origin. Obstruction causes distention of the bile ducts. The fibroblasts in the portal regions are stimulated to proliferate. The surface of the liver is finely granular. The liver cells often contain globules of bile which may become decolorized and then stain acidophilically.

### *Discussion*

(Dr. Virgil H. Moon, Philadelphia, Pa.) In the first place, I wish to express my deep appreciation of the presentation.

One thing I should like Dr. Mallory to answer, if he will, is whether he regards the hyaline reticulum which is so characteristic of alcoholic cirrhosis as due to the alcohol as a factor, or whether it is simply the earmark of that type in which alcohol as an etiological factor may be absent. I had opportunity to perform an autopsy on one of three cases occurring in children in the same family, in which that form of hyaline material in many cells was very prominent. The familial occurrence was a remarkable feature. The fact that these children had had streptococcus infections a few years previous was a factor to be considered, and the absence of any actual or presumptive use of alcohol is also a factor to be considered.

(Dr. Mallory, closing.) Years ago a doctor passing through Boston showed me a section of the liver containing hyalin from a child in Philadelphia, and promised to send me a section to keep, but I never received it. I would appreciate it if you would send me a section or some of the tissue from your case for study.

I am sure ethyl alcohol does not cause alcoholic cirrhosis. The name may be poor, but for the present it has to be used. What we are working for is the etiological factor. If I had to guess as to the cause of alcoholic cirrhosis, I should say in the first place that it is something which contaminates alcohol. Secondly, we usually have, in combination with the hyaline reticulum, fatty infiltration of the cells. If there is one thing which may cause the two conditions, fatty infiltration and the hyaline change, it is possibly chronic arsenical poisoning, which might possibly occur in children in connection with something they have taken in their food or drink. That is just a suggestion. I have been working on the problem for thirty-three years, and cannot find the cause. But I am sure it is not alcohol.

STUDIES OF ABNORMAL LIVER FUNCTION. George H. Whipple, Rochester, N. Y.  
*Abstract.* Bile salts make up an important constituent of the bile and the experiments to be reported indicate that these salts are formed only through the activity of the liver. When the liver is injured, the output of these bile salts may



EXPERIMENTAL COPPER POISONING. Frank B. Mallory and Frederic Parker, Jr., Boston, Mass.

*Abstract.* Copper is best administered by injecting subcutaneously a suspension of metallic copper powder in lard. The acute lesions, occurring within the first two or three weeks, are anemia, acute tubular nephritis, central or diffuse necrosis of the liver, hemoglobinuria, pigmentation of the liver, and sometimes of the kidneys. The chronic lesions consist chiefly of pigmentation of the liver with accompanying necrosis of scattered liver cells, of sclerosis which starts at the peripheries of the lobules where the pigmentation is always most marked, and of regeneration of liver cells diffusely and in small islands.

The early pigment granules in the liver stain quickly a deep blue in a slightly alkaline, aqueous solution of pure hematoxylin after fixation in alcohol. In this way they may readily be distinguished from the yellow pigment granules which may occur, sometimes in abundance, under natural conditions, in the liver of the rabbit and which do not stain with hematoxylin; while hemosiderin stains yellow to brown.

The blue staining of the pigment granules is evidently due to the presence of copper which disappears in the course of a few months, leaving behind hemofuscin granules which gradually change to hemosiderin. Both hemofuscin and the pigment granules occurring under natural conditions can be stained by basic aniline dyes.

STUDIES IN EXPERIMENTAL CINCHOPHEN POISONING. T. P. Churchill and F. H. van Wagoner (by invitation), Chicago, Ill.

*Abstract.* Dogs have been fed cinchophen in doses ranging from that used therapeutically to twenty-seven times that amount. About 80 per cent of these animals have shown one or more acute gastric ulcers. Areas of necrosis have been found in the livers of the dogs that have died to date. The animals have refused to eat food containing the drug for more than a few days at a time. During the periods of taking the drug there occurred an increasing retention of bromsulphalin and a decrease in blood urea. After several days of refusal to eat any food, bromsulphalin was again excreted in normal amount and the urea content of the blood rose to normal. This work is still in progress.

### Discussion

(Dr. Paul Klemperer, New York City.) Dr. Fishberg and I tried to produce liver changes in rabbits by feeding them Atophan, the Schering and Glatz preparation, and by giving it intravenously. Some of the animals died very shortly after the intravenous injection, so we could not expect them to show anything; other animals were given by mouth ten to twenty times the therapeutic dosage, and we were unable to find any definite change. I think Dr. Churchill's experiments also point in the same direction, that we must still be rather cautious in concluding that Atophan generally produces an acute liver atrophy. In the last three years we have observed three cases in which the clinicians were sure that the Atophan was the cause of subacute yellow atrophy. In these cases we did not see any gastric ulcer. We found marked duodenitis which is, however, often found in cases of acute yellow atrophy in which no Atophan was given.

(Dr. Howard T. Karsner, Cleveland.) I wish to make reference to some publications by Dr. H. S. Reichle, a former member of this Department, which dealt

one rabbits showed cirrhosis of the liver. When we consider that five of the twenty-one rabbits were fed copper for less than 30 days, it is evident that the percentage of pigmentation and cirrhosis is relatively high in those animals fed for 30 days or more. In the control rabbits practically no copper was found in their livers, while in the copper-fed animals there was found from 9.68 mg. to 237 mg. per 100 gm. of wet liver tissue. The copper-fed animals showing no pigmentation or cirrhosis were animals showing less than 100 mg. of copper per 100 gm. of liver substance. All but one animal that received copper for more than 40 days showed pigmentation, which in most cases was very marked. All of the animals receiving copper for more than 60 days showed cirrhosis of the liver. The animals were fed on their special diets for periods ranging from 21 to 105 days.

### *Discussion*

(Dr. Virgil H. Moon, Philadelphia.) I should like to ask if the authors of the paper have made an analysis, in human cases of hemochromatosis, for the presence of copper in the liver.

(Dr. Frank B. Mallory, Boston.) There has been a lot of work done in Aschoff's laboratory by Schönheimer and others. At first they were very enthusiastic about the presence of copper, but when they analyzed other cases of cirrhosis, they found an almost equal amount. I think the explanation is that the copper quickly disappears from the liver, and in these chronic cases it never has a chance to accumulate. It is not a cumulative poison, anyway. The copper disappears from its combination with hemoglobin, leaving hemofuscin. In two cases of very acute pigment cirrhosis with islands of regeneration, the islands, as I told you, at first contain no pigment, and then we get a pigment which gives a copper reaction. Fixed in formalin, the hemosiderin would not stain, except brown, while the copper stains blue. I have not gone far enough yet, but in these cases I get single cells and groups of cells which give a blue reaction (and that would signify that in the early stages copper was there), but in a few weeks it disappears.

(Dr. Hall, closing.) We have seen some of the acute changes pointed out by Dr. Mallory, and we agree with him in regard to them. Dr. Mallory has made it appear that it is extremely easy to demonstrate the presence of copper in these granules of hemofuscin. In 1928 I tried very hard to do this, for I thought there was copper in these granules, since the amounts of copper stored in the liver ran quite proportional to the amount of pigment present. Dr. Mallory has worked on this problem for ten years, and has only lately described a method for staining copper in the hemofuscin granules. I have also felt that there was more or less bound iron in the hemofuscin, and perhaps it may change to hemosiderin through oxidation. I have tried by means of incinerating the sections and oxidizing the bound iron to make it show up, but I think I lost it later in trying to stain it by the Berlin blue method. It might be possible to demonstrate it by Scott's method.

I wish to add that we fed three rabbits with their litter-mate controls on a diet containing sodium acetate for periods of 40 to 60 days, and we found no pigmentation. I am sure that this pigment that Flinn and VonGlahn found with sodium acetate is the natural pigment of the liver cells. We avoided carrots in our rabbits' diet, and we had none in our previous work, so it seems to me impossible that the pigmentation could be due to this factor of feeding carrots.

present time. It may be quite true, however, that many of the acute yellow atrophies of the early days had as their etiological agent some of these drugs.

It is very true that there is marked idiosyncrasy exhibited by the patient in the production of this disease. The clinicians have repeatedly noted that many patients consume large quantities of cinchophen without the slightest evidence of harm. On the other hand, certain individuals apparently are severely poisoned by even small doses. When the poisoning comes, its course is very rapid and jaundice a very early characteristic.

**STREPTOCOCCUS HEPATITIS.** H. Edward MacMahon and Frank B. Mallory, Boston, Mass.

*Abstract.* The more common inflammatory changes seen in the liver in cases of streptococcus septicemia are described and illustrated with cases that have come to autopsy.

Emphasis, however, is laid on a less common lesion of which three cases are given in detail, characterized by rather sharply circumscribed and irregularly distributed areas of liver tissue showing necrobiotic changes and necrosis, infiltrated with polymorphonuclear and endothelial leucocytes and showing masses of streptococci both intra- and extracellularly. This lesion — one of acute hepatitis — is ascribed to the toxic action of streptococci acting locally within the area involved.

The similarity of this lesion to the histological picture at times encountered in cases of acute yellow atrophy is discussed, and the suggestion is made that a careful search of the liver in the fixed preparation for streptococci, together with culture of the liver at the time of autopsy, might reveal bacteria within the lesions more commonly than is suspected — particularly in those cases of so-called acute yellow atrophy showing a very irregular distribution of the lesion — a condition that is extremely difficult to explain on the basis of a circulating toxin in the blood.

A fourth case is fully described showing a chronic inflammatory reaction within the liver in which one finds a picture of degeneration, necrobiosis and necrosis of liver cells, with dissociation of the intercellular reticulum, together with an active proliferation of bile ducts and connective tissue and a rather rich infiltration of endothelial leucocytes and lymphocytes. These changes are irregularly scattered throughout the liver, and in places have entirely replaced many lobules. Streptococci are numerous and confined to the lesions. A non-hemolytic streptococcus was recovered from the patient's blood during life, and a similar organism was isolated in pure culture from the liver at the time of autopsy. This case is presented rather for discussion than as a proved case of progressive cirrhosis of infectious origin.

The last point to be considered is the histological and gross changes which one may find in the healed stages of these acute and chronic inflammatory processes, and here again the suggestion is offered that certain types of cirrhosis, usually diagnosed as healed acute yellow atrophy of an atypical type (lesions that have been described as isolated cirrhotic nodules within the liver) may represent healed infectious hepatitis of bacterial origin.

The second part of the paper is devoted to results of experimental work. A streptococcus obtained from an early case of scarlet fever was injected, free of toxin, into radicles of the portal veins of both guinea pigs and rabbits. The animals were killed at varying intervals and the lesions produced, together with

with autopsies on patients who died of so-called toxic cirrhosis of the liver, in whose history there was the appearance of susceptibility to cinchophen.\* I recall not less than three of these cases, and in none of them was there an ulcer found in the stomach. Dr. Reichle continued this work on experiments with rats, and administered cinchophen in massive doses over long periods of time without discovering any lesion in the liver. He then attempted to injure the liver by chloroform treatment, and we assumed from controls that that injury was produced. Even after that injury, the prolonged administration of cinchophen had no effect. I think that Dr. Reichle's experiments lead us to assume that, as far as the rat is concerned, there is no liver injury produced by cinchophen, and from a survey of the whole matter I am inclined to say that, as far as man is concerned, the damage by cinchophen represents an idiosyncrasy.

(Dr. Churchill, closing.) I recall seeing those three cases that Dr. Karsner had reference to. I was at the City Hospital at the time. I do not mean to give the impression that I do not think Atophan does not produce liver damage; and the purpose of the paper is to produce acute yellow atrophy in the dog. However, none of those areas of necrosis was observed grossly, enough to cause very definite areas of necrosis in the gross pathology. They exhibited small yellowish areas, and the microscopic findings were the only ones where we found areas of necrosis at all.

THE SPECIFIC CHARACTERS OF TOXIC CIRRHOSIS AS OBSERVED IN CINCHOPHEN POISONING. A REVIEW OF FIVE FATAL CASES. D. C. Beaver (by invitation), and H. E. Robertson, Rochester, Minn.

*Abstract.* Attention is called to certain specific characters which these forms of cirrhosis possess. Particular attention is given to the types produced by anti-rheumatic medication, especially the cinchophen groups of drugs. Of these latter five fatal cases are reported. Attempt is made to correlate these cases with other types of so-called toxic cirrhosis and to differentiate them from the so-called atrophic, or Laennec's cirrhosis.

### *Discussion*

(Dr. Robertson.) It may be possible that the changes which have been described in this paper are not produced by cinchophen, and yet it is a remarkable fact that in several of these cases, when cinchophen was taken away from the patient, he became better and when it was given again he became worse and died. The lesions in these cases bear a certain resemblance to those of acute yellow atrophy, but their course is more chronic than acute yellow atrophy and still more rapid than the ordinary types of cirrhosis of the liver. Repair in these cases has more prominence than that of acute yellow atrophy, but less than the ordinary cirrhosis. There are all gradations, however, between the groups which we are accustomed to denominate acute yellow atrophy, cases of toxic cirrhosis such as those that are reported in this paper and which might be denominated subacute yellow atrophy, and the finished stages of so-called Laennec's or portal cirrhosis. I should like to be able to demonstrate that all of these types are related to a toxic destruction of the liver cells, but this hardly seems possible at the

\* Reichle, H. S.: Toxic Cirrhosis of the Liver due to Cinchophen. *Arch. Int. Med.*, 1929, 44, 281.

the very rapid destruction of these organisms by the fixed tissue phagocytes of the sinusoids of the liver. In such experiments as were done and reported in that article, I showed that the pneumococci were completely destroyed in 112 hours. The phagocytic cells do not infiltrate the capillaries, but are actual constituent cells of the capillary walls. These cells may be desquamated after they have phagocyted the pneumococci.

(Dr. Virgil H. Moon, Philadelphia.) I am interested in the presentation of this work, particularly as it duplicates some of the features that came under my observation not so long ago in a juvenile case of cirrhosis, having the gross features of Banti's disease or atrophic cirrhosis, in which cultures were made as described by Dr. MacMahon. A hemolytic streptococcus was obtained in pure culture from the substance of the liver. It was not obtained from postmortem blood culture, or from the blood during life. The same organism was obtained from the spleen. It was not found in the kidney. These facts indicate that it was not a terminal septicemia. Another case of similar character in which we had no opportunity to make cultures showed identical microscopic demonstration of the organism in the substance of the liver and of the spleen. An adult case of atrophic cirrhosis came to autopsy a few weeks ago. A pure culture of hemolytic streptococcus was obtained from the liver substance. The pathogenicity of this streptococcus is being studied at this time.

(Dr. MacMahon, closing.) I have been very much interested in the excellent work on infectious lesions of the liver already done by Dr. Moon.

Both cases which he has reported show extensive and long standing cirrhosis, and in both streptococci have been demonstrated; however, it is rather difficult to be sure exactly what part the streptococci are playing in such lesions of the liver.

In résumé, we believe that streptococci may produce an acute destructive inflammatory reaction within the parenchyma of the liver; that the organisms may be rapidly destroyed and removed from the areas of inflammation; and lastly there is evidence to show that the smaller foci may completely heal, whereas the larger areas probably result in isolated patches of cirrhosis.

GRANULOMATOUS ABSCESS OF THE LIVER OF PYOGENIC ORIGIN. D. C. Beaver  
(by invitation), Rochester, Minn.

*Abstract.* This is a study of eight cases of chronic pyogenic granulomatous infections of the liver which have a fairly distinct clinical and pathological picture. Their resemblance to actinomycotic infections of the liver is remarkable. Comparisons with actinomycosis and with more acute pyogenic abscesses of the liver will be presented.

CHANGES INDUCED IN THE LIVER BY MECHANICAL OBSTRUCTION OF THE  
HEPATIC VEINS. J. P. Simonds and (by invitation) J. W. Callaway,  
Chicago, Ill.

*Abstract.* The hepatic veins of dogs were mechanically obstructed by clamping off the hepatic veins by the method previously described by Simonds and Brandes. Careful asepsis was observed in these experiments and the animals were allowed to live for from 48 to 72 hours and then sacrificed. The following changes were observed in the livers of these animals: (1) scattered areas of marked passive hyperemia with disruption of the cords of liver cells; (2) edema and hemorrhage in the connective tissue about the sublobular veins; (3) areas of focal necrosis; (4) swelling and granulation of the cells in the central portions

the results of bacteriological studies, are described and compared with the lesions seen in human cases.

It should be pointed out very clearly that absolutely no attempt has been made to explain cirrhosis of the liver in its broad sense as a chronic or healed inflammatory process of infectious origin. On the contrary, the points that are particularly emphasized are just that an acute inflammation can occur in the liver as a result of the actual presence of streptococci within this organ; second, that with extensive destruction of liver cells, and the reparative proliferation of connective tissue and bile ducts, wide tracts of sclerosis can be produced — giving a picture which resembles in many respects the healed stages of acute yellow atrophy.

### *Discussion*

(Dr. Stuart Mudd, Villa Nova, Pa.) I should like to ask if this finding of phagocytosis by the endothelial leucocytes rather than by the polymorphonuclears is in general characteristic of streptococcus hepatitis.

(Dr. MacMahon.) In the first case reported the reacting cells were almost entirely endothelial leucocytes. In other cases also showing the acute lesions, polymorphonuclear leucocytes were equally as numerous. In answer to Dr. Mudd's question, in our cases phagocytosis of the streptococci has been limited to the endothelial leucocytes.

(Dr. Alfred Plaut, New York City.) In the cases reported a focus could be demonstrated as the source of infection. In case an infant lived for a longer time, it might happen that this demonstration would be no longer possible. Only two weeks ago I did an autopsy on an infant 30 days old. I found streptococcus peritonitis, and an enlarged liver which in the gross looked exactly like the liver of congenital syphilis — so much so that I started to hunt for spirochetes in spite of the absence of any other gross signs. No spirochetes were found. The peritoneum gave a pure culture of streptococcus. The liver microscopically contained a meshwork of necrosis which went irregularly through the whole liver. The umbilical vessels, examined in cross-section from five different planes, were entirely normal, the degree of occlusion corresponding to the age of the infant. Later on I saw the physician who had seen the infant before it came to the hospital, and he told me the child had had a typical septic infection of the umbilicus. I wonder what might happen to a liver if an infant should survive such an infection.

(Dr. MacMahon.) This is the type of case we are primarily interested in, one showing an acute destructive inflammatory lesion of the parenchyma. It would be interesting if streptococci could be demonstrated within the more recent lesions of this liver. Even if organisms could not be demonstrated, streptococci should not be definitely excluded, because streptococci acting locally can produce considerable damage, and in a very short time may be killed and completely removed.

I have not reviewed the literature regarding the changes that one would find in the liver had the child recovered, but I have found cases reported showing isolated foci of cirrhosis of unidentified origin. We have also seen livers both in children and in adults showing isolated patches of cirrhosis. Some of these cases may be explained on the assumption that at one time there was an infectious lesion which had subsided.

(Dr. Preston Kyes, Chicago.) Some fifteen years ago I published a paper, the conclusion of which was that the resistance of birds to pneumococci was due to



of the lobules; (5) phagocytosis of erythrocytes and excessive hemosiderin pigmentation of Kupffer cells; (6) hyaline thrombi in many central and sublobular veins, and (7) the presence of masses of cells occluding the sinusoids similar to the masses observed in dogs that have survived anaphylactic and peptone shock.

## READ BY TITLE

ASSIMILATION OF ATLAS AND COMPRESSION OF MEDULLA: WITH CONTRIBUTION TO THE CLINICAL SIGNIFICANCE AND PATHOLOGY OF TORTICOLLIS AND LOCALIZED CHRONIC ARTHRITIS DEFORMANS OF THE SPINE. Istvan Bezi (by invitation), Budapest and Rochester, N. Y.

STUDIES ON THE ADSORPTION OF DIPHTHERIA TOXIN ONTO AND ELUTION FROM MAGNESIUM HYDROXIDE. E. E. Ecker and (by invitation) L. A. Weed, Cleveland, O.

AN INVESTIGATION OF SPIROCHETOSIS OF THE DENTAL ANLAGE IN CONGENITAL SYPHILIS. T. J. Hill (by invitation), Cleveland, O.

THREE FATAL CASES OF BACILLUS PYOCYANEUS INFECTION. B. S. Kline and (by invitation) A. S. Maschke, Cleveland, O.

THE PATHOLOGY OF BARTONELLA MURIS ANEMIA AND THE COMPENSATORY PHENOMENA FOLLOWING SPLENECTOMY IN THE ALBINO RAT. J. Marmors-ton-Gottesman and David Perla, New York City.

ACUTE ENCEPHALOMYELITIS OF THE HORSE. EPIZOÖTIC IN THE SAN JOAQUIN VALLEY, 1930. K. F. Meyer, San Francisco, Calif.

URINE FLOW AND OPEN GLOMERULI AFTER UNILATERAL NEPHRECTOMY. R. A. Moore and (by invitation) W. W. Summerville, Cleveland, O.

X-RAY SARCOMA OF MAN WITH REPORT OF A CASE. Frederick W. Mulsow, Cedar Rapids, Iowa.

MYOCARDIUM IN SYPHILIS. Otto Saphir, Chicago, Ill.



however, found instances reported of primary dissecting aneurysms involving the pulmonary, lineal, thyroid and the cerebral arteries.

In a vast majority of cases the intima is lacerated at some point. The tear is most often located in the ascending portion of the aorta. Secondary rupture is frequent and usually occurs through the adventitia. Depending upon what portion of the adventitia is perforated there is hemorrhage into the pericardial sac, mediastinum or pleura. Sometimes the adventitia is ruptured in several places with the production of multiple hemorrhagic phenomena. Secondary rupture back into the lumen of the aorta has been observed in a number of cases, but this is relatively infrequent. The extent to which the aorta may be involved by the aneurysm varies from a few centimeters at one side of the base to complete separation of the coats throughout the circumference and length of the vessel, with extension down the iliac arteries. It is in this extensive type that reperforation into the lumen of the aorta is most frequently seen. In these extensive processes the large branches at the arch of the aorta and in the abdominal portion may be involved to a varying extent. The intercostal and lumbar vessels are frequently torn across or may remain intact and traverse the aneurysm. The clinical manifestations of a blockage of the aortic branches have been stressed by Crowell.

The fate of those suffering from dissecting aneurysms is usually death by hemorrhage, as indicated above. Occasionally the aneurysms which have a double opening from the aorta "heal." That is, the aneurysmal tube becomes endothelialized with the formation of a double aorta (Hall, Bostroem, Rindfleisch, Cleland, Adami, MacCallum, etc.). This is, of course, a rarity, some fifty cases having been found by Resnik and Keefer in 1925.

Laennec considered atheromatous change of the intima and its primary rupture to be the cause of the disease. Peacock attempted to produce dissecting aneurysms by injecting water into the aorta of cadavers. He found that the lesion could not be produced in normal aortas simply by heightened pressure in the vessel lumen. When the intima was damaged, however, dissection occurred if the rent in the intima was transverse to the long axis of the vessel. He concluded that a dissecting aneurysm could not be produced in a healthy vessel. In his cases of dissecting aneurysm Rokitansky, in 1852, found two distinct types of alteration in the vessel. In one the adventitia was

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## DISSECTING ANEURYSMS \*

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New Haven, Conn.)*

### INTRODUCTION

The first description of a dissecting aneurysm is usually accredited to Laennec in 1826, but Peacock states that Mannoïr recognized the lesion in 1802. The condition was certainly not generally appreciated until Laennec's publication, and since this time an extensive literature has grown around the subject. In 1925 Resnik and Keefer found 300 cases reported in the literature up to that time.

The pathogenesis of dissecting aneurysms is still in dispute and the conception of the mechanism of their formation has undergone a gradual evolution. The earlier authors were divided into two schools. The first thought that trauma to the vessel (high blood pressure) was the essential factor, with or without a pathological process in the coats of the vessel. The second considered disease of the intima to be of paramount importance with high blood pressure as a secondary agent. Later, attention was centered on changes in the medial coat, but the nature of these changes and the mechanism of rupture were debated, although the majority of authors agreed that intimal rupture was produced by high blood pressure acting in the presence of a weakened media. Within recent years a few individuals have stated that in some instances the medial changes cause hemorrhage into the aortic wall from the vasa vasorum, with secondary rupture of the intima.

The commencement of dissecting aneurysms is practically limited to the aorta, its branches being involved secondarily. Loeschke,

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the media. These processes caused the layers of the media to separate more readily and it is their contention that this increased separability is a necessary forerunner of dissecting aneurysms. Moriani also observed minute hemorrhages from the vasa vasorum in the media which he thought coalesced and caused the dissecting aneurysm. He also found fatty and hyaline changes in the media resulting in an increased separability of the medial fibers and concluded that medial rupture preceded the intimal tear.

Shennan and Pirie concluded that dissecting aneurysms are caused by vessel changes of two types. In the first the rupture is due to the presence of intimal atheromata. This type of aneurysm is limited in extent since it early breaks through the adventitia and results in death. The second variety is due to pathological changes in the middle and outer thirds of the media. The elastic strands are fragmented and more friable and, due to their rupture, small rents are produced which are filled with homogeneous material. The connective tissue is more abundant and shows hyaline and fatty changes. The muscle tissue is atrophic and decreased in amount. In the adventitia the alterations are not marked although there is an increased amount of connective tissue. The vasa vasorum are thickened and have either mononuclear or polymorphonuclear cells about them. These authors concluded that in this type of dissecting aneurysm the primary rupture is of the media, followed by the intimal tear.

In one case Krukenberg found three dissecting aneurysms, one extending the whole length of the aorta and starting from an intimal tear at the mouth of the innominate artery. The others were in the right and left inferior thyroid arteries, in one of which the intima was intact throughout. This author found inflammatory changes in the media similar to those marked by Babes and Mironescu and believed that in some dissecting aneurysms the medial changes are of primary importance. He pointed out that a tear in the intima is not necessarily an essential feature of dissecting aneurysms and held that in those cases in which the intima is intact the aneurysm undoubtedly arises from rupture of the vasa vasorum. He also believed, with Shennan and Pirie, that in the type of dissecting aneurysm which arises from the vasa vasorum the intimal tear is secondary to the production of the dissecting aneurysm and occurs through an atheromatous patch, as do all reperforations of such aneurysms into the vessel lumen.

thickened, abnormally vascular and more easily separated from the media. In the other instance degenerative changes were present in the media. This coat was brittle and separated easily from the adventitia.

Since Rokitansky most authors have emphasized the medial changes as being of paramount importance, although nearly all agree that high blood pressure and primary rupture of the intima are essential factors in the actual production of the aneurysm. A wide variety of changes has been described. Thus von Recklinghausen observed minute tears in the media not associated directly with the aneurysm, and thought that the dissection occurred because of invisible (molecular) alterations in the physical properties of the tissue. Bostroem concluded that trauma is the most important factor in the production of dissecting aneurysms whether disease of the media is present or not. Degeneration of all the elements of the media in a 20 year old man was observed by Brouardel and Vibert. Degenerative changes in the media, unassociated with the disease process in the intima, were observed by Voigts. These changes involved particularly the elastic lamellae.

Lebert was the first to find inflammatory changes in the aorta associated with dissecting aneurysms. He found mononuclear infiltration and new-formed connective tissue cells in the adventitia and outer media. The elastic lamellae were destroyed and the vessel wall was weakened. Inflammatory medial changes also were observed by Lüttich but in his case the intima was markedly thickened while the media was atrophic, and this author concluded that dissecting aneurysms result from the intimal changes. Manchot found similar medial changes but concluded that they were secondary to the aneurysm and that laceration of the medial elastic fibers was the primary cause of the disease.

Inflammatory overgrowth of the muscle cells and areas of necrosis in the media were observed by Tschermak. The necrotic areas resulted in progressive thinning and final rupture of the elastica. In addition to these changes, new vessel formation and round cell infiltration were present. The whole length of the aorta was involved, but the lesion was particularly pronounced at the arch. This author applied the term *mesarteritis* to the condition.

Babes and Mironescu found moderate cell and vessel proliferation followed by minute hemorrhages, all the changes being confined to

together with the findings of a high blood pressure and failure to concentrate the urine, pointed toward renal disease, and a diet was prescribed. From this time on the patient was seen at frequent intervals on the Medical Service of the New Haven Hospital. On her first visit she had a slightly elevated blood non-protein-nitrogen, a blood pressure of 230/148 and a decreased plithalein output. The urine showed a trace of albumin and the specific gravity varied between 1.005 and 1.010. Early nephritic retinitis was present. During the next five years the patient was admitted to the New Haven Hospital seven times. The blood pressure remained above 200 systolic and the retinal lesions progressed so that central vision in both eyes was lost. At the age of 49, two years before her death, the signs of cardiac failure became manifest and from then on frequent digitalization was necessary. The final admission was at the age of 51 when she presented herself for digitalization. At this time the blood pressure was 265/140, cardiac hypertrophy was pronounced and the eye grounds showed extreme changes. The non-protein-nitrogen of the blood had reached 76 mg. per 100 cc. The blood Wassermann was negative, as it had been throughout her course. Digitalization was only partially successful and eleven days after admission she suddenly experienced difficulty of speech and in a few minutes had a complete right-sided hemiplegia. Consciousness was retained. The blood pressure rose to 295 systolic and the apex impulse of the heart was forceful. About forty minutes after the onset of paralysis she suddenly became cyanotic and in a minute or two was unconscious and gasping for breath. The apex beat and pulse at this juncture were impalpable and the heart sounds were inaudible. In another minute the heart stopped beating and the respirations ceased after a few more gasps.

*Pathological Observations:* The body weighed 60 Kg. and measured 166 cm. in length. The pericardial sac was distended with 425 cc. of clotted and fluid blood. The epicardium over the base of the aorta was lifted up by an accumulation of clotted blood. The base of the aorta was encircled by this hemorrhagic process which also extended up the aorta as high as the attachment of the epicardium, where it stopped abruptly in its upward course. The sub-epicardial hemorrhage extended laterally from the aorta to encircle the pulmonary artery. The reflection of the epicardium on the pulmonary artery was broken through, and the blood had extravasated into the loose tissue around the pulmonary artery and its branches to the hilum of each lung, and also extended anterior to the trachea over the arch of the aorta and along this vessel for a distance of 10 cm. beyond the origin of the left common carotid artery. The intimal lining of the thoracic division of the aorta showed slight atheromatous changes, there being a few yellow plaques scattered along this portion of the vessel. The vessel, however, was inelastic and brittle. The abdominal portion showed advanced atheromatous changes and some calcium deposition. The intima bulged inward at

Whitman and Stein reported a case where the medial layers of the aorta were split apart and a collection of clear fluid filled the defect. The intima of the vessel was intact. These authors laid great stress on the medial changes they found which were similar to those described by Babes and Mironescu and by Krukenberg.

Bay's case was remarkable because of a great decrease in the amount of elastic tissue of the aorta with increased brittleness of the vessel, which suggested to him the presence of a special disease process. This observer pointed out that whereas syphilis and arteriosclerosis may be causative in some instances, the occurrence of dissecting aneurysms in young people (boy of 8 years — Rokitansky) shows that these processes are not the only agents. Bay believes that raised blood pressure and blood vessel changes involving principally the elastica are necessary factors. He also pointed out the frequency of chronic nephritis concurrently with dissecting aneurysms.

Von Schnurbein stated that the underlying cause of dissecting aneurysms is Thoma's disease of the vessel and that the split occurs at the point of deepest penetration of the vasa vasorum into the vessel wall.

One case was presented by Loeschke where an undoubted gumma was present. He stated that syphilis is an unlikely cause unless definite necrosis is present.

Uniform changes were found by Lifvendahl in three cases. These consisted of an intimal tear at the base of the aorta, high blood pressure, renal arteriosclerosis and syphilitic mesaortitis. In one case a rheumatic vascular lesion could not be excluded. The syphilitic process was always most marked at the site of the intimal tear. This investigator reported the finding of syphilitic mesaortitis in one case each by Uhles and by Gsell, and the finding of renal arteriosclerosis in conjunction with dissecting aneurysms by Oppenheim, Busse and Loeffler. He stated that dissecting aneurysms occurred during labor in five cases cited by Bohmen.

## CASE REPORTS

**CASE 1. *Clinical History:*** The patient, a white female, had never been a robust individual. In childhood she had measles, whooping cough and pneumonia. Since puberty severe frontal headaches occurred with each menstrual period. At the age of 31 the left breast was removed because of the presence of a benign tumor. At 46 years of age the patient entered the New Haven Hospital for a tonsillectomy and at this time the history of weakness and blurred vision,

slit remained. In the outer media numerous vessels which did not show proliferative changes had a broad zone of hemorrhage about them, splitting apart the medial fibers. Many of these vessels were unassociated with the aneurysm but others were seen to lead into it. Occasional areas of perivascular fibrosis were encountered and sometimes the scarring dipped in from the adventitia. A few vessels had a narrow cuff of mononuclear leucocytes about them. Throughout the media, but particularly in the inner two-thirds, the collagenous tissue was increased in amount and appeared to replace the muscle fibers. The elastic lamellae were not decreased in number. They showed some fragmentation, but the most characteristic change was an irregular broadening out of the strands so that the fibers had a jagged appearance with an ill-defined border. Fat was abundant and was most plentiful in the inner third of the media. Minute fat droplets were present in the central part of the media adjacent to elastic lamellae, apparently replacing muscle tissue.

*Comment:* The patient had had definite and progressive renal disease for at least five years. The blood pressure during this time had been constantly elevated, usually above 200 mg. of mercury. At autopsy advanced generalized arteriosclerosis with particular involvement of the renal and cerebral vessels was present and the heart was greatly hypertrophied. Evidence of syphilitic mesaortitis was lacking, but the vasa vasorum at the base of the heart showed marked intimal proliferation. Many vasa vasorum were completely obliterated while others were thickened. Some were torn and had a zone of hemorrhage about them. Arteriosclerotic changes in the intima of the aorta were slight, whereas the media showed advanced degenerative changes. The hemorrhage was confined to the medial and adventitial coats and was apparently a terminal event owing to the early development of hemopericardium.

*CASE 2. Clinical History:* A white woman of 45 years was brought to the Accident Room of the New Haven Hospital with a diagnosis of perforated gastric ulcer. She had had influenza in 1918. Six years before admission she began having intermittent attacks of pain in both legs which forced her to remain in bed for four to five days at a time. For the past two years occasional attacks of paroxysmal dyspnea had been present. The seizures lasted about one-half hour. She had had dyspnea on exertion for many years and recently there had been some edema of the ankles. On the day before admission to the hospital she had fainted after slight exertion. She regained consciousness in about ten minutes and vomited a small amount of green material. She was brought to the hospital the next morning, having been suffering with great pre-

the base of the aorta on the left, but the lining was intact throughout the entire vessel. After multiple cross-sections had been made to include the aorta, pulmonary artery and the base of the heart it was found that there was a large hemorrhage in the wall of the aorta which had split apart the fibers of the middle and outer thirds of the media and caused the inward bulging of the intima. The hemorrhage began 1.5 cm. above the aortic cusps and extended to within 1.5 cm. of the innominate artery. Above the aortic valve the hemorrhage half encircled the aorta but tapered to a point at its upper limit. Four centimeters above the aortic cusps the outer third of the media and the adventitia had ruptured and allowed the hemorrhage to extend beneath the epicardium on the base of the aorta. Two small tears were found in the epicardium behind the right auricular appendage. These tears permitted the development of the hemopericardium.

The heart weighed 675 gm. The right ventricle measured 6 mm. in thickness while the left measured 20 mm. The papillary muscles and trabeculae carnae of the right ventricle were flattened. Each kidney weighed 52 gm. and each showed the nodular, scarred surface associated with arteriosclerotic change.

The cerebral arteries showed advanced sclerotic changes and a massive hemorrhage practically replaced the left cerebral hemisphere.

Microscopic preparations of the ascending portion of the aorta showed that the middle and outer thirds of the media were split apart by hemorrhage. Red blood cells were packed between the medial fibers immediately surrounding the hemorrhagic area. There was no exudate present, however, and no evidence of necrosis, although some of the fibers stained less intensely than those further removed from the aneurysm. The adventitia was denser than usual and this layer and the subepicardial tissue over the base of the heart were packed with red blood cells which showed a typical thrombotic structure in many areas. The intima was slightly thickened and its lamellated structure had been replaced by accumulations of mononuclear leucocytes and amorphous material typical of early atheromata. The most striking changes were found in the vasa vasorum. In the adventitia and outer third of the media most of these vessels had undergone intimal proliferation which was so extensive in some cases that the lumen was entirely obliterated, while in others a mere



with blood. The epicardium had two minute perforations in its anterior surface between the aorta and the right auricular appendage. This allowed the blood to escape into the pericardial sac. On section of the lungs it was found that the blood followed the branches of the pulmonary artery on each side for a considerable distance.

In the microscopic preparations the break in the media was found to be a horizontal slit with irregular edges. The space between the severed medial fibers was filled with blood and the free edges of the muscles and elastic bundles were rolled up. Red blood cells were packed between the separated fibers for a short distance around the laceration but no leucocytic exudate was present and, although the tissue at the edge of the tear was stained poorly, there was no definite evidence of necrosis. Distally the hemorrhage extended to the origin of the innominate artery and lay between the intima and media, the former coat being intact throughout. Near the heart the blood had dissected between the outer and middle thirds of the media. At the base of the heart the blood lay nearer the adventitia. At the attachment of the aorta to the heart the hemorrhage extended entirely beneath the adventitia. About 1 cm. above the base of the aorta the adventitia was broken through and the hemorrhage had spread out into the subepicardial tissue.

The base and ascending portions of the aortic wall showed marked degenerative changes. In the adventitia and outer media the vasa vasorum had greatly thickened walls, so that some of the vessels were entirely occluded, while others were nearly so. The media was greatly distorted, particularly in the first 8 to 10 cm. of the vessel. There was but little muscle tissue left. Much of it was replaced by irregular accumulations of an amorphous, blue-staining material histologically resembling mucus. Many of these areas contained fat droplets. The amount of collagenous tissue was increased. There was a great decrease in the number of elastic lamellae and those which remained were short and frayed. There were numerous irregular, clear spaces or rents in the media which frequently occurred in the neighborhood of the vasa vasorum. Between the base and arch of the aorta there were many vasa vasorum which had ruptured and allowed red blood cells to extravasate into the surrounding tissue. In a few instances a vessel was surrounded by a narrow band of mononuclear leucocytes. The intima presented a normal appearance except in a few places where there were atheromatous thicken-

cordial pain and increasing dyspnea throughout the night. On admission the temperature was 101° F., the pulse was 100 and the respirations 32 per minute. The blood pressure was 140/80. Cyanosis was marked. The heart was found to be greatly enlarged and signs of early consolidation were present at the base of the right lung. The liver was palpable five finger-breadths below the costal margin. The blood Wassermann was negative. Eight hours after admission the patient died suddenly while sitting up in bed. About twenty hours had elapsed since the onset of symptoms.

*Pathological Observations:* The body was obese and weighed 72 Kg. The important findings were limited to the thoracic contents. The pericardial sac was distended by an accumulation of about 350 cc. of clotted and fluid blood. At the base of the heart and aorta the epicardium was bulged out by an accumulation of recently clotted blood. With this clot the organ weighed 700 gm. Most of the increased weight was due to hypertrophy of the left ventricle, the walls of which measured 22 mm. in thickness, while the right ventricle was 3 mm. thick. None of the valves displayed noteworthy changes. Throughout the aorta the endothelial lining of the intima was intact. There were numerous yellow plaques present, none of which contained calcium. The sclerotic changes were particularly marked in the descending thoracic and abdominal portions where there were numerous plaques about the orifices of the vessels. Two centimeters above the opening of the right coronary artery was a deep puckering of the intima and to the left of this the intima appeared to be stripped up from the media. On section through the posterior wall of the aorta it was seen that the media was lacerated transversely 3 cm. above the base of the aorta. The tear extended 2.5 cm. to the left of the intimal puckering described above. Extending upward from the laceration the intima was dissected away from the media for a distance of 4.5 cm. The space between these two layers was filled with clotted blood. Below the transverse tear the blood dissected its way between the media and adventitia. Just over the base of the aorta posteriorly there was a small perforation of the adventitia which allowed the blood to escape beneath the epicardium. The subepicardial space was bulged out with clotted blood over the base of the heart, extending as high as the pericardial reflection on the base of the aorta. The subepicardial hemorrhage extended between the aorta and pulmonary artery and surrounded the latter structure and its branches to the hilum of each lung. The mediastinal tissues around the tracheal bifurcation were infiltrated

vations, some of which were calcified. Between the origins of the innominate and the left common carotid arteries there was an aneurysmal outpouching 2 cm. in diameter which was entirely filled by a firm, laminated thrombus. The wall which bounded the aneurysm was continuous with the aortic wall. The entire intimal lining of the aorta was intact throughout. Each kidney weighed 150 gm. and they both had finely granular surfaces. The pelvic fat was increased in amount. Arteriosclerosis was advanced throughout all the peripheral vessels.

Microscopically there was definite evidence of syphilitic meso-arteritis. This reaction was most pronounced at the attachments of the aortic cusps and in the wall of the saccular aneurysm between the innominate and left carotid arteries. The lesion was active in these areas and was characterized by the presence of numerous capillaries and a heavy infiltration of mononuclear leucocytes. This reaction was present in the adventitia and media and in a few instances extended to the intima. There was considerable necrosis of the medial fibers in the region of the aneurysm and throughout the syphilitic zones there were large accumulations of yellow pigment indicative of old hemorrhage.

As in the preceding cases the vasa vasorum of the adventitia and outer media were partially or completely occluded by subintimal proliferation. Some of the vasa vasorum of the media were torn and red blood cells had extravasated about them. In the entire ascending portion of the aorta the elastic lamellae were swollen, fragmented and decreased in number. This was particularly noticeable in the areas which showed definite syphilitic changes. The muscular component of the media was largely replaced by collagenous tissue and it was noteworthy that the bundles did not run in definite rows but were irregular, giving a cross-hatched appearance to the sections. In some instances the medial fibers stained poorly, were free of nuclei and appeared necrotic. This reaction was seen as a sharply demarcated band which partially encircled the aorta in the outer and middle thirds of the media. It was seen most clearly in the region where the aneurysm had dissected the medial fibers apart. The intima was thickened and showed considerable atheromatous change. Sections of the kidneys demonstrated numerous thickened arterioles with scarred kidney substance alternating with hypertrophic changes in the unscarred portions.

ings. In the sections from other parts of the body there was marked intimal proliferation in the arterioles. There was slight passive congestion of the liver and a few vascular scars in each kidney.

*Comments:* In this case there was a dissecting aneurysm present in a woman of 45 years. The intima was intact throughout but the walls of the aorta were split apart by hemorrhage. The media showed marked degenerative changes. The vasa vasorum were thickened or obliterated. Many were torn, forming small intramural hemorrhages.

*CASE 3. Clinical History:* While talking to his employer the patient, a white male of 70 years, suddenly fell, sustaining a laceration in the occipital region. He was brought to the New Haven Hospital by ambulance and was unconscious upon arrival. He died before an examination could be made. It was ascertained from relatives that the patient had suffered from shortness of breath for the past six months and that he had tired easily. Four months before death he had consulted a physician, who, it is stated, found a totally irregular heart action and signs of cardiac failure. The blood Wassermann was negative at this time.

*Pathological Observations:* At autopsy, seven hours after death, the body was found to be well nourished and well developed. It weighed 63 Kg. and measured 163 cm. in length. The pericardial sac was distended by an accumulation of 500 cc. of clotted and fluid blood. The heart weighed 400 gm. The coronary arteries were tortuous, thickened and brittle. Numerous atheromatous plaques were present in the intima but the vessels were patent throughout. The heart valves showed no noteworthy changes. The subepicardial tissue over the base of the aorta and the pulmonary artery was distended with clotted blood. The hemorrhage extended along the branches of the pulmonary artery into the hilum of each lung. There was also considerable hemorrhage into the mediastinal tissue. One centimeter above the base of the aorta posteriorly there was a tear in the epicardium which allowed the blood to escape from the subepicardial space into the pericardial sac. There was a massive hemorrhage between the media and adventitia of the aorta which began at the attachment of the aorta to the heart and extended upward for a distance of 7 cm. beyond the origin of the left subclavian artery. The hemorrhage lay between the media and adventitia throughout most of its extent, but in a few places the middle and outer thirds of the media were split apart. These tears led into the adventitial coat. The intima throughout the entire aorta was involved in extensive atheromatous processes which produced discreet and confluent ele-

There was a dissecting aneurysm which involved the entire length of the aorta from the base to the bifurcation. Throughout its length the aneurysm lay between the outer coats of the media. At the base of the aorta the entire vessel was encircled by the process, which gradually narrowed until at the arch about one-half of the circumference of the aorta was dissected. At the arch the aneurysm lay posteriorly, and this relation was continued to the bifurcation. Some of the intercostal and lumbar vessels were torn across at their origins while others were intact. There was extensive hemorrhage into the adventitia over the base and ascending portions of the aorta. This hemorrhage followed the pulmonary arteries to each lung and spread out into the mediastinal tissue. The adventitia was ruptured beneath the epicardial reflection on the base of the aorta and the subepicardial tissues were distended with blood. Between the right auricular appendage and the aorta there was a tear in the epicardium 3 mm. in length which evidently allowed the hemopericardium to develop.

Serial sections through the region of the intimal tear demonstrated that the dissection extended almost straight through the media to the adventitia. The medial coats were separated in their middle and outer thirds and this level of dissection was maintained almost constantly throughout the aorta. Around the edge of the intimal laceration the medial fibers were split and the spaces were infiltrated with red blood cells. Along the line of longitudinal dissection the medial fibers were rolled up and there was abundant hemorrhage between the medial fibers adjacent to the aneurysm. In the ascending portion and arch of the aorta the outer third of the media was broken through in many places, with resultant hemorrhage into the adventitia.

The most striking changes were in the vasa vasorum and the fibers of the medial coat. The vasa vasorum of both adventitia and media showed the same intimal proliferation and reduction in the size of the lumina as has been described in the previous cases. In the media many vasa vasorum were torn and had hemorrhages about them. There was no definite histological evidence of syphilitic mesaortitis.

*Comment:* The dissecting aneurysm occurred in a 54 year old man who had serological, but no definite histological evidence of syphilis. The most uniform changes in the aorta were seen in the vasa vasorum, many of which were thickened and obliterated while

*Comment:* The case was one of sudden death from hemopericardium in a 70 year old man with a six months' history of cardiac disease. The heart was hypertrophied and although no reading was obtained it is probable that the blood pressure was elevated. The dissecting aneurysm occurred almost entirely between the media and adventitia although the media was involved to some extent. Syphilitic mesaortitis was present and in addition advanced degenerative changes had taken place in the medial coat. As in the other cases the vasa vasorum were extensively diseased, some having decreased lumina while others were torn and had old and recent hemorrhage about them.

*CASE 4. Clinical History:* The patient was a white male of 54 years. He was brought to the New Haven Hospital in a semiconscious condition. Three hours before admission he had had a sudden sharp pain in the back and slight numbness of the left leg and arm. He was cyanotic and the skin was cold. Great difficulty in breathing was experienced and there was a marked sensation of precordial oppression. Six months before there had been a similar attack with headache, dizziness, cyanosis and numbness of the left leg. The attack lasted three days. At this time the patient's doctor told him that he had a high blood pressure.

On admission to the hospital the patient was semicomatose but coöperative. Extreme cyanosis of the face and neck was present, the neck veins were engorged and the respirations were slow and shallow. The pulse and blood pressure could not be obtained. The heart sounds were weak and distant. The deep reflexes were absent. The patient vomited twice. Twitching of the left arm was noted and death took place forty-five minutes after admission to the hospital and about three hours after the onset of the illness. The Wassermann taken shortly before death was 2 plus in the alcoholic and the cholesterinized antigens.

*Pathological Observations:* The body was well developed and well nourished, weighed 79 Kg. and measured 169 cm. in length. The pericardial sac contained 590 cc. of clotted and fluid blood under some pressure. The heart weighed 600 gm. The left ventricle averaged 20 mm. in thickness while the right averaged 8 mm. in thickness. The coronary arteries were not unusually tortuous and the vessel walls were not thickened. There was a tear in the intima of the aorta which began 1 cm. above the right posterior sinus of Valsalva and extended upward in a semicircle for 3 cm. to end on the anterior wall of the aorta. There was extensive hemorrhage in all three coats of the calcified vessel about the tear. There was an atheromatous ulcer 2 cm. in diameter near the mouth of the innominate artery. Otherwise the intima was free from such change.

aorta to the arch the dissecting aneurysm was filled with clotted blood but from here on the aneurysmal walls were in close apposition. At the commencement of the aorta, just above the valve cusps, there was a 2.5 cm. tear in the intima which was transverse to the long axis of the vessel. The upper edge of the tear overhung the lower to a slight extent and both edges were ragged and injected. The intima, throughout the ascending and transverse portions of the aorta, was discolored a dark, diffuse red with some yellow, raised, atheromatous areas showing through. The mouths of the intercostal vessels showed a considerable amount of sclerotic change. Otherwise the intima was quite smooth. The entire vessel, however, was brittle and it was observed that the edges of the aneurysm could, by slight manipulation, be extended around the aorta.

Multiple cross-sections were made of the entire aorta. It was observed that 5 cm. above the base the outer third of the media and the entire adventitia were ruptured, allowing blood to dissect between the media and adventitia and also beneath the epicardium, leading to hemopericardium through rupture of the epicardium, as described above. The great vessels at the aortic arch were split for varying distances by the hemorrhage, the innominate artery being the most extensively involved.

Seen microscopically, the base of the aorta was greatly decreased in thickness and the tissue was distorted. The intima was apparently the least damaged layer, although here there was some hyaline change and in places there were partially disintegrated red blood cells between the strands of tissue. The decrease in thickness was at the expense of the media where the muscle fibers were broken and replaced by hyalinized connective tissue over wide areas. What scanty elastic tissue remained was broken and frayed. There were many large gaps in the medial tissue filled with homogeneous acellular material which stained blue in hematoxylin and eosin preparations. There were also many clear spaces in this part of the vessel which were empty and represented tears in the wall of the vessel. Sections across the tear at the base of the artery demonstrated that the tear proceeded in an almost straight line through the intima and inner media to the outer fifth of the media. Here the medial fibers and the adventitia were thrown into outward curving folds by the pressure of blood. In the loose, subepicardial tissue around this point there was a dense cellular reaction consisting of an infiltration

others were torn, giving rise to intramural hemorrhage. The medial fibers showed advanced degenerative changes.

*CASE 5. Clinical History:* The patient was a hod-carrier, 49 years old, who, while at work, suddenly experienced severe substernal pain. He became dizzy, dyspneic and lost consciousness for a few minutes. He left work and when he reached home intense pain was present in the epigastrium, which radiated down the inner side of both thighs. A doctor was called who found that a flaccid paralysis of both legs was present and that there was a loss of sensation from the symphysis pubis to the feet. Large doses of morphine failed to relieve the pain. Cyanosis appeared and death occurred about four hours after the onset of symptoms.

*Pathological Observations:* The body was well developed and nourished, weighing 90.9 Kg. There were 50 cc. of bloody fluid in the peritoneal cavity. There were a few fibrous adhesions at both pulmonary apices and calcified nodules were present in the tracheal nodes. The parietal pericardium was infiltrated with dark red blood throughout its anterior surface. The pericardial vessels were clearly demarcated coursing through the hemorrhage. The pericardial sac contained 350 cc. of clotted and fluid blood. The pericardial reflection on the base of the great vessels was lifted up by an accumulation of blood which entirely surrounded these vessels as far as the arch of the aorta and along the pulmonary artery into the hilum of each lung. The angle of reflection of the epicardium on the aorta was broken through, allowing blood to infiltrate the pericardium and extend in the loose tissue to the arch of the aorta. There was a tear in the epicardium 0.5 cm. in length, situated between the aorta and pulmonary artery. This evidently permitted the development of the hemopericardium.

The heart and aorta were removed intact and fixed in 10 per cent formalin. The heart weighed 600 gm. The left ventricle was greatly hypertrophied. All valve structures were within normal limits. The aorta was opened along its anterior surface. It was seen that there was a separation of the coats of the vessel throughout its entire length. The separation occurred between the layers of the media. At the base of the aorta the separation encircled four-fifths of the circumference of the vessel. At the arch the vessel was split about two-thirds of the way around, and from the arch to the bifurcation one-half of the circumference was involved. The intercostal arteries were torn across as they traversed the aneurysmal sac. The proximal lumbar vessels were similarly affected. From the base of the



twenty hours, and in all instances was due to hemopericardium. The lowest cardiac weight was 400 gm.; the rest of the hearts weighed 600 gm. or more. In three cases the intimal lining of the aorta was intact. Two were lacerated just above the aortic cusps. The aneurysm extended from the base to the arch in all but two cases and in these two the entire vessel was involved down to the bifurcation. The path of dissection of the aneurysm was nearly always between the middle and outer thirds of the media. Occasionally the plane lay between the media and adventitia but only for a short distance. Even in those cases where the intima was lacerated the tear proceeded straight through the media to its outer third before pursuing a longitudinal course. The pulmonary vessels and mediastinal tissues were infiltrated and in all cases the outer media and adventitia were ruptured at some point beneath the reflection of the pericardium on the base of the aorta. This led to the development of hemopericardium through perforation of the epicardial layer over the base of the heart.

The intimal lining of the thoracic portion of the aorta was surprisingly free from change in all but one case which showed numerous atheromata. Striking changes were observed in the media of all cases. Here degenerative processes were paramount and were particularly marked in the ascending portion. The muscle fibers were decreased in number, and fat and collagenous tissue replaced them. The elastic fibers were apparently fewer and many of those which remained were broken and frayed. In many instances there were broad zones where the medial tissue had lost its cellular outline and was apparently composed of hyalinized tissue. Jagged rents in the continuity of the media were of frequent occurrence. Small accumulations of material, histologically resembling mucus, were seen packed between the muscle and elastic fibers. Fat deposits as small droplets were present as far out as the outer third of the media.

The most pronounced and uniform change was in the vasa vasorum of the ascending aorta. In the adventitia and loose areolar tissue just outside this coat these vessels showed the most advanced intimal thickening, resulting in some instances in complete occlusion, while the lumen of others was reduced to a mere slit. In the outer half of the media, in addition to being thickened, many of the vasa vasorum were torn and had zones of hemorrhage about them. This process was noted at a distance from the path of the dissecting

of endothelial leucocytes, lymphocytes and a few polymorphonuclear leucocytes. Fibroblastic activity was also present. The dissection proceeded up the aorta and gradually assumed a position between the middle and outer thirds of the media. This level was maintained constantly throughout the rest of the vessel. Throughout the aorta the media was extensively changed. The elastic tissue was decreased in amount; the muscle fibers were poorly developed and there were many areas composed of fibrous tissue. The vasa vasorum showed considerable intimal proliferation and reduction in size of their lumina. It was further observed that there was a reduction in the total number of vessels at the base of the aorta. Many vasa vasorum were surrounded by an infiltration of mononuclear leucocytes. There were but few instances of hemorrhage surrounding the vasa vasorum. The tissue adjoining the line of the dissecting aneurysm was not more extensively changed than the rest of the media.

*Comment:* A 48 year old laborer without history of previous heart disease died of a dissecting aneurysm extending the whole length of the aorta. A tear was present in the intima just above the aortic cusps. The most striking change was the degeneration of the medial coat at the base of the aorta. The vasa vasorum did not show the obliterative changes described in the previous cases and the intima was relatively free from disease. A heavy cellular infiltration was present in the adventitia over the base of the aorta and was most marked opposite the intimal tear.

#### SUMMARY OF CASES

In the five cases presented the oldest was 70 years of age, while the others ranged between 45 and 54 years of age. In the first four cases where adequate histories were obtained there is evidence of pre-existing cardiac disease, the duration varying from a few months to over two years. The onset of illness was characterized in all five instances by the sudden appearance of precordial pain or oppression, dyspnea and prostration. Loss of consciousness accompanied the onset in four individuals. In the fifth case the blood pressure was not determined but in the others a reading was taken either before or during the attack and was found to be high. The blood Wassermann was positive in one instance (Case 4). After the onset of symptoms death occurred at intervals varying from a few minutes to

changes are paramount. The highest pressure in the lumen of the aorta occurs at the base; therefore it is at this point that the adventitia finally gives away with the subsequent development of hemopericardium. Laceration of the intima must also be considered in the light of the foregoing discussion. The old statement that high blood pressure and an intimal tear, however produced, are necessary and sufficient factors for the production of a dissecting aneurysm is hardly tenable. It is more certain that medial degeneration is a prerequisite and it is probable that intimal laceration is secondary to the formation of the dissecting aneurysm in most, if not in all, cases.

There are several facts which bear out this point of view. In the first place the reasoning employed to explain the rupture of the adventitia would apply to tearing of the intima as well, since it is nearly always at the base of the aorta that the intima is lacerated. When other sites are chosen it is usually possible to find a deep atheromatous process extending into the media which would sufficiently weaken the intimal lining to allow perforation to occur from the aneurysm in the media through to the aortic lumen. Secondly, in the usual case with the intimal tear occurring just above the aortic cusps, the break proceeds straight through the vessel to the outer media and never strips up the intima. Furthermore, a third point to be considered is the fact that over the surface of the ordinary atheromatous ulcer the intimal lining is often deficient, yet no dissection of the vessel coats is observed around the ulcer. Finally, Gallavardin and Gravier observed a case where the intima was lacerated transversely just above the aortic cusps — the favorite site in dissecting aneurysms — and no dissection of the coats occurred. They state that the media was normal histologically.

Determining etiology from the study of histological sections is always dangerous and in searching for the underlying cause of the medial changes occurring in these cases one can go no further at present than the lesions observed in the vasa vasorum. The occlusion of large numbers of these vessels might easily so embarrass the blood supply of the media that degeneration of this coat would result.

The presence of syphilitic mesaortitis in one case substantiates, rather than vitiates the argument, since this disease notably attacks the vasa vasorum with resulting intimal proliferation, and also involves the media, with the production of necrosis in the active phase

aneurysm and also near the aneurysm and leading into it. In all cases many of the intact vasa vasorum had a cuff of mononuclear leucocytes about them. In one instance there was undoubted evidence of syphilitic mesaortitis and the process was fairly active.

## DISCUSSION

The outstanding feature of the cases reported here, and of many of those abstracted from the literature is the uniformity of the pathological process. The majority of cases occur in patients between 45 and 60 years of age, although the aorta is not markedly sclerosed in these individuals, as judged by the extent of intimal change. The entire vessel, however, is inelastic and brittle. As would be supposed from the blood pressure determinations, all the cases had hypertrophied hearts. The base of the aorta is first affected and is the portion most extensively involved by the aneurysm. The path of dissection followed by the aneurysm is constant with but a few minor variations, and the mechanism of death in each case is practically identical. The similarity is further emphasized by microscopic study of the aorta, when it is seen that the media, particularly at the commencement of the vessel, is the seat of advanced changes. These consist of degenerative processes which reach such a high degree that the media must be greatly weakened. The fact that in this series three of the dissecting aneurysms occurred without a break in the intimal lining does away with the common conception that an intimal tear is a necessary factor in the production of the disease. Also, in these cases the vasa vasorum obviously are the only possible source of the hemorrhage.

A plausible explanation of the mechanism for the rapid development of a dissecting aneurysm which is not in communication with the aortic lumen is sought. Owing to the weakened condition of the medial coat, plus the heightened blood pressure, one or more vasa vasorum rupture into the medial coat. The hemorrhage forms an intramural hematoma. The internal pressure in the aorta is exerted upon the hematoma and causes it to spread along the line of least resistance, *i. e.*, the media, since this is now the weakest coat and, as it has been demonstrated, its fibers are more easily separated. The intramural hemorrhage begins at the base of the aorta and attains its greatest size at this point, since it is here that the medial

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and fibrosis late in the course. Syphilis as the chief cause of dissecting aneurysms is unlikely, since in a majority of the cases in the literature this lesion was absent and in four of the present series there was no evidence of the disease histologically.

A further possibility is suggested by Case 5. In this instance the vasa vasorum at the base of the aorta did not show the marked occlusive changes that would be expected from the high degree of degeneration observed in the media. In fact no vasa vasorum at all were visible over large areas. There was, however, a chronic inflammatory process in the subepicardial areolar tissue over the base of the aorta. This area coincided with the commencement of the aneurysm and it is possible that the inflammatory process had completely destroyed the vasa vasorum. This case would, therefore, represent a more advanced stage in the diminution of the vascularity of the aortic coats.

### CONCLUSIONS

1. The development of a dissecting aneurysm of the aorta is apparently dependent upon degenerative changes in the medial coat.
2. The underlying cause of the medial change is probably obliteration of a large number of vasa vasorum from arteriosclerosis or a low grade inflammatory process.
3. The aneurysm begins by a rupture of one or more vasa vasorum into the weakened medial layer, with the formation of a hematoma which splits apart the medial fibers.
4. A tear in the intima of the aorta is not a necessary factor in the formation of a dissecting aneurysm.
5. When initial tears do occur, they are probably secondary to the development of the aneurysm.

NOTE: The author wishes to express his thanks to Dr. Raymond Hussey for his interest in this work and for permission to publish the cases.

## DESCRIPTION OF PLATES

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### PLATE 125

FIG. 1. Dissecting aneurysm splitting apart the medial fibers on the posterior wall of the aorta. The inward bulging of the intima is due to a large hematoma in the media. Slight atheromatous change. The outer media has ruptured allowing blood to extravasate beneath the adventitia of the aorta and pulmonary artery. Drawing from Case 2.

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## PLATE 126

FIG. 2. Dissecting aneurysm between the middle and outer thirds of the media. The outer media of the aorta has ruptured, causing a massive subadventitial hemorrhage completely surrounding the base of the aorta and pulmonary artery. The intima shows advanced atheromatous changes but is intact. There is a syphilitic, saccular aneurysm between the innominate and left carotid arteries. The fibrous cord of the ductus arteriosus is present. Drawing from Case 3.



PLATE 127

FIG. 3. Dissecting aneurysm between the fibers of the media. The intima shows slight atheromatous change, but a laceration is present just above the aortic cusps. The outer media has ruptured, allowing blood to extravasate beneath the adventitia around the aorta and pulmonary artery to the hilum of each lung. Drawing from Case 4.

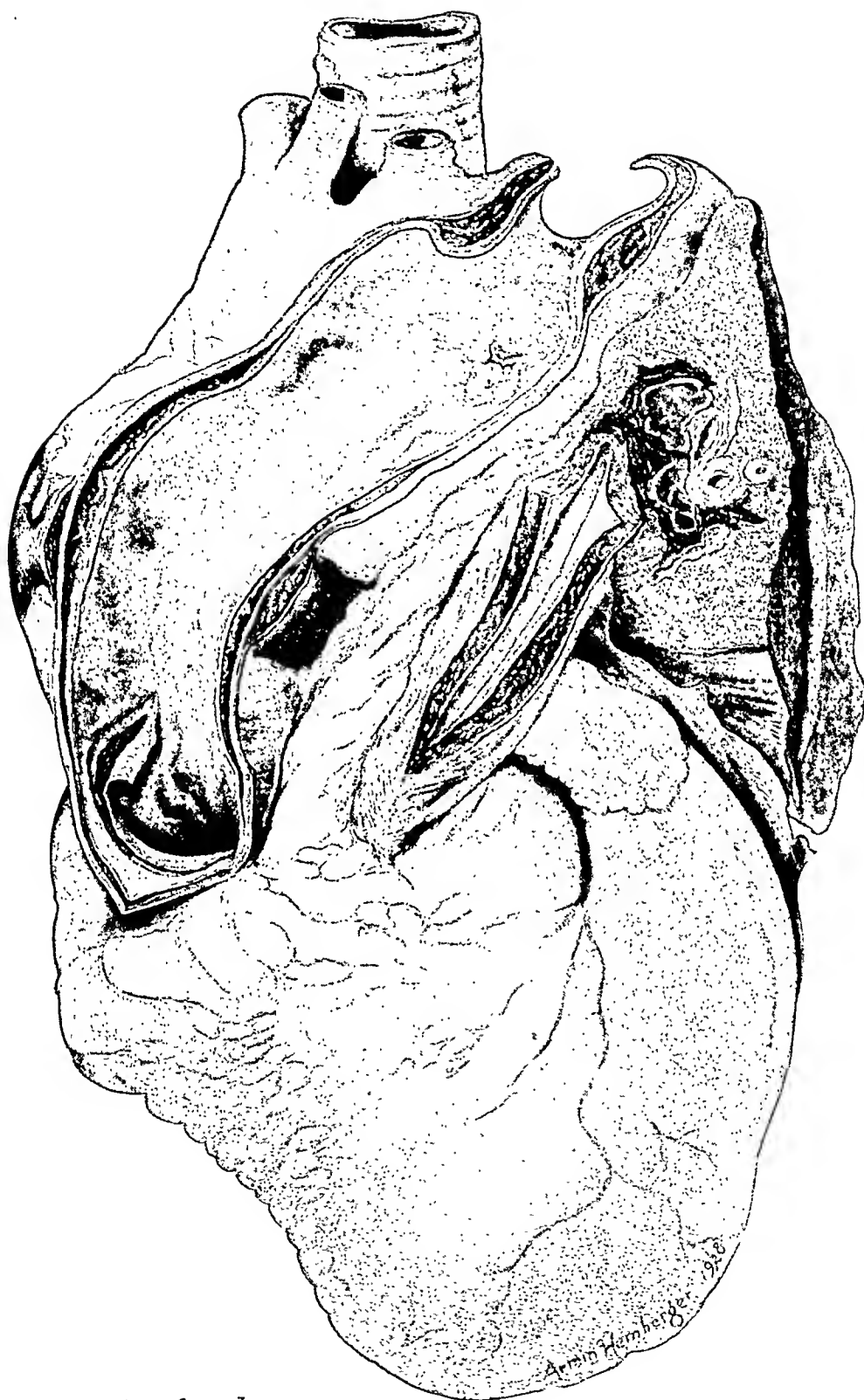


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PLATE 128

FIG. 4. Dissecting aneurysm extending the whole length of the aorta with a laceration of the intima just above the aortic cusps. The intimal lining is discolored by the blood outside it. Slight atheromatous change is present. The media has ruptured, allowing blood to extravasate beneath the adventitia. The adventitia has ruptured beneath the epicardium and this layer in turn has perforated at a point between the origins of the aorta and pulmonary arteries, causing hemopericardium. Drawing from Case 5.



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## PLATE 129

Cross-sections of the aortas from the cases described, showing the path of dissection of the aneurysm with hemorrhage in the tissue surrounding the aorta.

FIG. 5. At the level of the innominate artery. Case 5.

FIG. 6. At the level of the innominate artery. Case 4.

FIG. 7. At the level of the common carotid artery. Case 5.

FIG. 8. At the base of the heart. Showing rupture of outer third of media with hemorrhage beneath adventitia. Case 1.

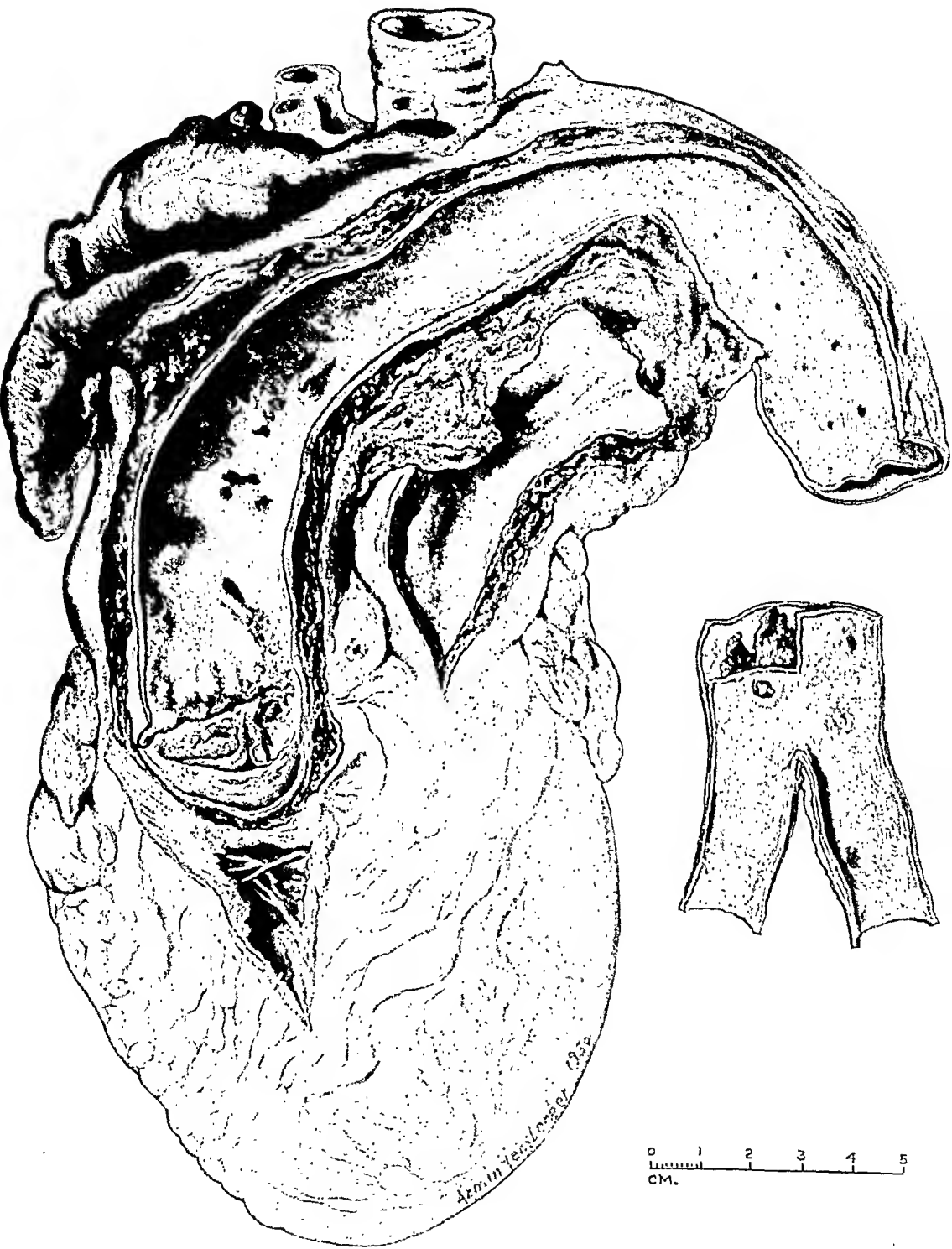




PLATE 130

- FIG. 9. A section at the base of the aorta showing the laceration of the intima and media with splitting apart of the media and adventitia by hemorrhage. Case 5. Hematoxylin and eosin stain.  $\times 20$ .
- FIG. 10. Large vas vasis in the adventitia at the base of the aorta. Occlusive changes are marked and the lumen is represented by a mere pin-point opening. Case 2. Hematoxylin and eosin stain.  $\times 85$ .
- FIG. 11. Arteriole and vein in the outer media. The arteriole is nearly occluded by thickening of its wall. Massive hemorrhage is present in the adventitia. Case 1. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 12. Rupture of vas vasis of media with extravasation of red blood cells. Case 1. Hematoxylin and eosin stain.  $\times 125$ .



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PLATE 131

- FIG. 13. Extravasation of red blood cells around vasa vasorum of media. Case 1. Hematoxylin and eosin stain.  $\times 120$ .
- FIG. 14. Two thickened vasa vasorum deep in the media. Degenerative changes about them. Case 4. Hematoxylin and eosin stain.  $\times 120$ .
- FIG. 15. Syphilitic changes at base of aortic cusp. Mononuclear infiltration and new vessel formation. The dark spots are composed of changed blood pigment indicative of old hemorrhage. Case 3. Hematoxylin and eosin stain.  $\times 120$ .
- FIG. 16. Hemorrhage in media of aorta at the top of the picture. Large spaces or "rents" in media at bottom of picture. Loss of nuclear structure and hyalinized appearance of medial fibers. Case 4. Hematoxylin and eosin stain.  $\times 120$ .
- FIG. 17. Large irregular tear in media with marked retrograde changes in the medial fibers. Some small round cell infiltration is present. Case 3. Hematoxylin and eosin stain.  $\times 50$ .
- FIG. 18. Section completely through the aorta at the base. The entire wall of the vessel is thin and atrophic. The normal structure is absent. Most of the wall is occupied by large spaces filled with homogeneous blue-staining material. Only a few distorted muscle and connective tissue fibers remain. Case 5. Hematoxylin and eosin stain.  $\times 50$ .



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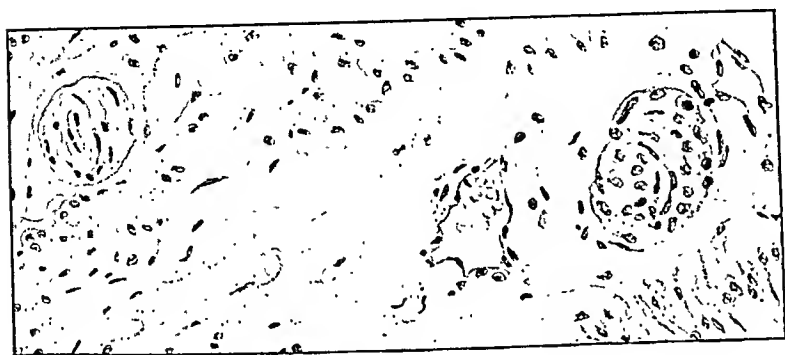


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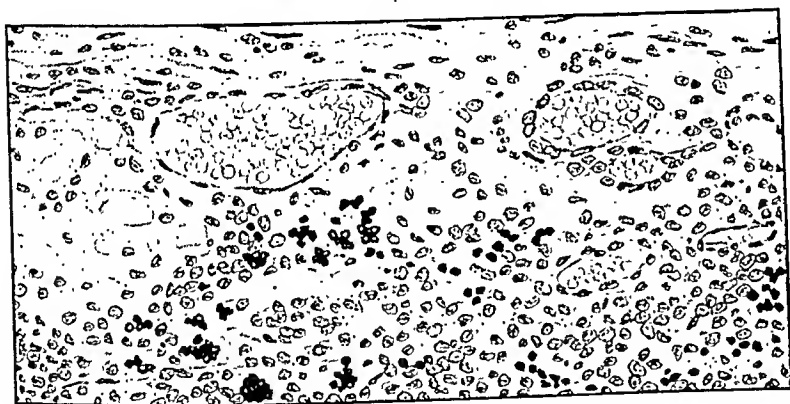
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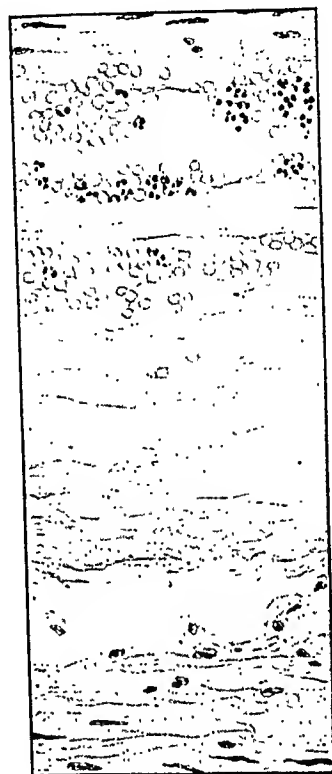
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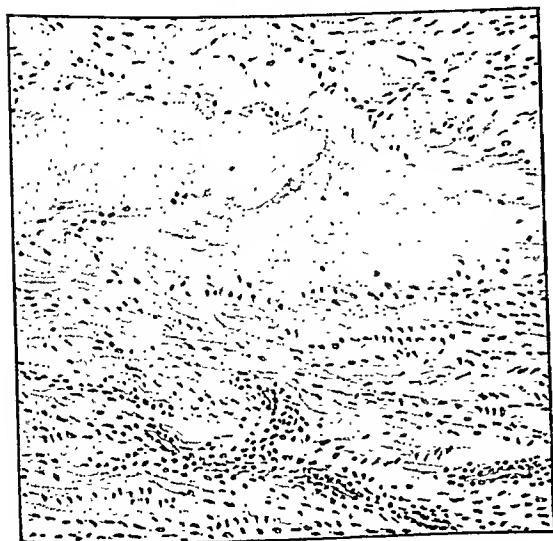
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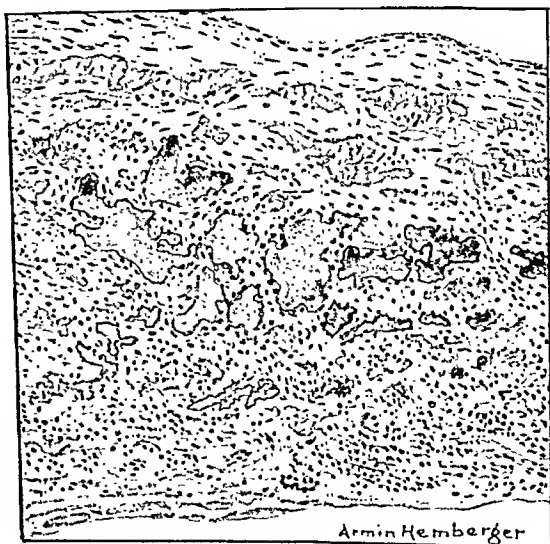
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Armin Hemberger

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A monograph of 232 pages by Dawson,<sup>7</sup> which appeared two years prior to Miescher's article, should not be overlooked in a discussion of the melanomas. In this excellent paper, which is very profusely illustrated, Dawson considers every side of the question from all angles but one, the neurogenic, which was not brought into prominence until Masson rescued it from the discard. Dawson's point of view is, therefore, necessarily an epidermalistic one. In considering the tumors said to be primary in the nervous system, melanoma of the meninges, and so on (pp. 671-672), he expresses doubt as to their being true melanomas, but he discusses them with an open mind and refers to the literature that explains their origin on a chromaffine basis. For a complete review of the subject up to 1925, and as a source of reference material on melanoma (there are seven pages of bibliography), this article will be found to be invaluable: it is probably the most complete exposition of this topic in the English language, taking up the consideration from the historical, developmental, normal histological, pathological and clinical points of view, and presenting many valuable points in the way of classification.

Four origins have been assigned to the melanoma: skin, endothelium, connective tissue and the tactile corpuscles of Wagner-Meissner. The dermal and nervous origins have come into increasing prominence and we may neglect the others for the present. Masson's expansion of Soldan's theory is as follows: melanoma originates in the cells of the Wagner-Meissner corpuscles, in the more peripherally situated Merkel-Ranvier bodies, and in scattered cells of a similar nature embedded in the epidermis. All of these are situated along the course of sensory nerve endings, which are at first myelinated and lose their sheaths as they enter the cell groups of the corpuscles, to terminate in arborizing or reteform plexuses with varicose nodes around the epithelioid cells. Thus the nerve fibers and their cells play a part in these tumors, giving them a resemblance to neurofibromas. Building up this thesis Masson begins with the cell of the tactile apparatus as the type cell and derives therefrom two forms of benign tumor: (1) the superficial or quiescent nevus of the epidermis and (2) the deeply situated, pigmented nevus of the derma. From these the transition to the malignant forms ("melanosarcoma," "melanocarcinoma") is readily traced. The type cell has two potentialities: it may be non-pigmented and associated with

## TWO CASES OF MELANOMA OF THE MENINGES WITH AUTOPSY \*

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### INTRODUCTION

Scarcely a dozen cases of primary melanoma of the meninges have been reported, so that the condition is by no means common and further details, accumulated from carefully studied material, should be of value, both as proof of the potential meningeal origin of this tumor and as evidence for the theory that melanoma is of nervous rather than of dermal origin.

That melanoma is of nervous origin and is closely related to neurofibroma was the thesis of an article by Soldan<sup>1</sup> in 1899. Masson<sup>2, 3</sup> resurrected this theory from the oblivion into which it had fallen when he wrote his excellent articles nearly thirty years later. He says that he had already come to his conclusions before discovering that Soldan had antedated him, but although he gives Soldan priority, the credit for putting the nervous origin of the nevi and melanomas on a firm and convincing basis, and for discovering their connection with tactile corpuscles, belongs wholly to Masson.

It is needless to cite all the literature on the subject; the reader will presumably be interested merely in knowing the recent developments in the matter. It is only today that Masson's articles, written five years ago, are beginning to bear fruit. In this country this is chiefly due to the fact that Ewing<sup>4, 5</sup> has recognized the merits of his claims and sponsored them in the latest edition of his book "Neoplastic Diseases," as well as in a brilliant paper in the *British Medical Journal*. In spite of this, however, the older views continue to flourish and we find Miescher<sup>6</sup> (who published a lengthy article on the dermal origin of melanoma about the same time that Masson's papers appeared) adhering to the dermal theory, although cognizant of Masson's work which he mentions as being praiseworthy, ingenious, but not proved.

\* Received for publication June 21, 1931.



prickles and fibrils and becomes associated with its fellows into cell nests that are quite unlike epidermis. Unfortunately, as Ewing points out, the unquestionable association of epidermal proliferation with the production of nevi and melanomas (also seen in other subepidermal conditions), the questionable histogenesis of the Langerhans melanoblast and the occasional presence of epidermal "pearls" in melanomas, all appear to bear out this process of reasoning. In this theory no account is taken of the nervous framework of the tumor, the nerve endings in the cell nests, the proliferation of myelinated nerve fibers near the base of the tumor, or of the presence of the small, lymphocytoid cells that Masson interprets as cells of the sheath of Schwann — all of which he points out in detail. This is due to the fact that stains have been employed which fail to bring out most of these details. Masson stresses the futility of attempting to rely on the routine stains in this connection, in an appendix to his second article.

Which of these two theories puts the least strain on one's imagination? Is it easier to accept the elaborate anaplasia and subsequent metaplasia of the epidermalists' theory, or the straightforward, systematic derivation of the tumor from wayward nervous elements of the tactile apparatus, as advanced by Soldan, Masson and Ewing?

In this paper, it will be our purpose to describe two cases in which melanomas originated in the meninges and not in the skin, to report the results of a careful study of these tumors by means of a very varied technique and to endeavor to adduce therefrom further proof as to the validity of the theory of nervous origin of melanoma. There is no evidence in either of our cases of the existence of pigmented tumors in the skin or the eye. We admit in advance that such tumors may have been present, but as none was noted at autopsy, or in the clinical histories, this seems improbable. Cerebral symptoms dominated the picture in both instances, the eyegrounds were examined in both and nothing was found, aside from choked discs in one case. That melanoma can and does arise from the meninges is accepted by the best authorities. In that case, how could it arise from epidermal cells? One would have to postulate all sorts of unlikely possibilities: inclusion, fetal rests and the like. In support of the neurogenic origin of such growths we have the statement of histologists, Maximow among them, that many meningeal

nerve endings, or it may be pigmented more or less deeply and apparently less intimately associated with them in that case. The first type is the "nevus cell," the second the "melanoblast." As proof of this theory Masson sets forth the following points: (1) these tumors have a readily demonstrable neurofibromatous substratum; (2) they have the structure of distorted Wagner-Meissner corpuscles; (3) with his trichrome stain he demonstrates medullated and non-medullated nerve fibers among the tumor cells and the marked similarity of these to those of the Wagner-Meissner and Merkel-Ranvier bodies, and (4) silver impregnation, which he admits was imperfectly carried out, bears out his ideas as to the presence of nerve fibers and filaments. The whole theory is beautifully presented and leaves little to be done in order to prove its validity, aside from carrying out more perfectly executed silver impregnations.

The melanoblast, which gives the tumor its name, Masson believes to be a phase of the type cell in which melanin metabolism plays the chief part; this, he thinks, is secondary and incidental. The branched or racquet-shaped melanoblasts are regularly intercalated in the basal cell layer of the epidermis and have given rise to the theory of basal cell origin of the melanomas. They are also known as "Langerhans cells." Their histogenesis has long been a topic of lively dispute. Whatever it may be, Masson remarks: "*Les cellules de Langerhans sont*" — they exist. The pigmented melanophores of the derma he believes to be ordinary histiocytes that have taken up melanin dropped by the melanoblasts.

The "epidermalist" school, typified by Bloch and Miescher, explain the tumor as follows. Somewhat naïvely orienting their theory to the conventional microscopic section of skin, with the epidermis at the top and the derma at the bottom, they speak of a loosening of the basal cells, with a dropping-down ("*Abtropfen*") into the derma in plugs or columns in the manner of stalactites, or scattered discretely. This theory is quite familiar to us all. Accordingly as the origin of these pigmented basal cells is assigned to epidermis, connective tissue, or adventitia, we derive three of the four histogenic theories. The epidermalist recognizes at once the fact that the type cell differs from the epidermal cell in several respects: it is clear, not cloudy; it lacks intercellular bridges or "prickles"; it has no intracellular fibers and it differentiates into two types of cell in the tumor. To explain this he must postulate that the cell rounds up, loses its

brain stem and the fissure of Sylvius. On attempting to strip the pia from the brain, some of these nodules were found to extend into the cortex for a distance of several millimeters and were inseparably connected with the brain tissue. Most of them were flat and barely palpable, and in the gross they appeared much like old petechial hemorrhages. The cerebral convolutions were markedly flattened. On sectioning the brain, a dark red mass, about 2.5 cm. in diameter, was found in the area just above the quadrigeminal plate, apparently obstructing the aqueduct of Sylvius and connected with the meninges and choroid plexus in this region. The third and lateral ventricles were dilated, and minute discolored areas, somewhat similar to those in the meninges, were found on their walls.

*Microscopic Examination:* Routine examination of sections from the brain and cord revealed tumors, apparently originating in the mass in the neighborhood of the choroid plexus and third ventricle (which was the only sizable growth found), or arising in a multiple fashion. They were composed of closely packed ovoid cells that frequently contained melanin and were more or less perithelial in their grouping. They were not unlike the usual acinar "melanocarcinoma" in their appearance. Usually superficial in the meninges, they sometimes invaded the cortex, particularly in the case of the cerebellum which showed columns of tumor cells penetrating quite deeply into its substance. The pigment was examined for iron, lipins and melanin, and the tests proved it to be the last. Reticulum stains showed a copious reticular network in the tumor and its metastases, the fibrils being apparently derived from the meninges.

*CASE 2. Clinical History:* White male, 45 years of age. The patient was admitted to the hospital in delirium and rapidly lapsed into coma. The only history obtainable was that he had complained to his family physician of headache, dizziness and vomiting six weeks prior to admission. A complete neurological examination was not made at the time. Three weeks later, following the extraction of several teeth, his symptoms became more pronounced and whenever he attempted to walk, he staggered toward the right. Two days before admission he became delirious.

Physical examination showed a well developed, well nourished, unconscious man whose heart, lungs, abdomen and extremities were essentially unremarkable except for the following reactions: Brudzinski's positive bilaterally; abdominal negative; triceps questionable; cremasteric positive only on the right; Babinski's, Oppenheim's and Gordon's present bilaterally; contralateral Gordon and Oppenheim on the left; Kernig's positive bilaterally. The ocular fundi showed markedly choked discs, but were otherwise normal. There was cervical rigidity. The spinal fluid was clear, but under increased pressure. The white count was 21,000, the temperature 98 F. All other findings were negative. It

nerves end in organs quite similar to the Wagner-Meissner corpuscles. What more could the proponents of this theory desire?

In our cases, then, we must show the complete similarity between our meningeal tumors and ordinary epidermal melanomas and we should prove the absence of skin or ocular lesions. The former we can do; the latter we can assume for the reasons already given: to the best of our knowledge and belief no such tumors were present.

## REPORT OF CASES

**CASE 1. *Clinical History:*** A white male, about 40 years of age, was admitted to the hospital complaining of severe headache that had been intermittent, with gradually increasing intensity since its sudden onset about one month before. The pain was most severe over the vertex. About two weeks prior to admission projectile vomiting, unassociated with nausea, followed eating. The patient's wife had noticed a change in his character during the last few weeks, which she described as "sort of delirium with increasing irritability and carelessness in personal habits." For the last three years the patient had been a diabetic, the disease being under dietary control. His past and family history were irrelevant to the present illness.

Physical examination revealed a well developed, well nourished man, disoriented as to time and space and showing slow cerebation. There was marked exophthalmos, but the pupils and fundi were normal. The right drum membrane was slightly congested, but not bulging. The knee jerks and Achilles' reflexes were absent; Brudzinski's and Kernig's signs were positive. All other physical signs were irrelevant. The spinal fluid was bloody, under increased pressure, with positive globulin, negative Wassermann and only fourteen cells. Clinical and chemical blood tests were negative, save for a white count of 10,800 and a blood sugar of 130 mg. Temperature was 99 F, blood pressure, respiration and pulse rate essentially normal.

The patient was under observation and treatment in the hospital for eighteen days before his death, during which time his left leg became spastic, ataxic and finally weak. There was increasing drowsiness with decreasing ability to coordinate and to cerebrate, dysphagia and dysphasia developed and finally stupor supervened, the extremities became weak and cyanotic and the patient died about seven weeks after the onset of symptoms. No definite diagnosis was made before death: the impression was hemorrhagic encephalitis.

***Autopsy:*** Three hours postmortem. The body and extremities were essentially normal externally and internally, with the following exceptions: there was an early lobular pneumonia, congestion of the abdominal viscera and moderate obesity.

When the meninges were exposed, numerous small, smut-colored nodules were seen to be scattered through all portions of the pia arachnoid of both brain and cord. They varied from pin-point to 3 mm. in size and were most numerous over the frontal lobes, the

ternally and in the fissure of Rolando. There was little hemorrhage into this nodule and it appeared to be a younger growth than the larger one in the temporal lobe. The brain tissue of the right cerebral hemisphere showed evidence of compression and there was almost complete collapse of the right lateral ventricle. No other pathological changes were observed in the brain. On account of the fact that brain tumors do not ordinarily metastasize to the lungs, all the viscera, as well as the external surface of the body and its internal membranes, were examined a second time for a possible primary focus, but none was found.

*Microscopic Examination:* This revealed the brain tumors to be exactly similar to one another (Fig. 3). They were composed of solid nests of ovoid cells with a delicate nucleus, prominent nucleolus and clear to cloudy or granular cytoplasm. In this case also, as in Case 1, the perithelial grouping was striking. It was only after having discovered melanin in some of the cells of the lung tumors that we went back and found a few melanotic cells in the brain nodules. They were essentially amelanotic in appearance, until silver impregnation picked out many latent melanin granules (Fig. 4). A reticulum impregnation revealed copious reticulum in the larger brain tumor, scanty reticulum in the smaller, similar in its arrangement to that seen in Case 1. The lung metastases, for we must consider them as such, showed essentially the same picture. They differed in that there was more melanin, although it was scanty at best, and found only in certain areas of the slides. A further difference was a tendency for the tumor cells to form hollow acini, quite similar to those of an adenocarcinoma, between which aberrant cells were scattered in the dense reticular stroma. With the Morgan-Weigert iron-hematoxylin method it was seen that the cells lining these acini often projected quite to the middle and formed a rosette-like body with their "tails" radiating toward the center. In other places the cells were layered and more or less concentrically arranged, as in the Wagner-Meissner corpuscles; in fact, the resemblance of some of the pulmonary tumor acini to these structures was very striking.

#### SPECIAL STAINS AND IMPREGNATIONS

Rather than go into the structure of the tumor any further, from the standpoint of ordinary methods of staining, it would be better to detail some new pointers toward its nervous origin. Reasoning that

was believed that the patient had either a brain abscess or tumor; therefore an exploratory craniotomy was done and a needle inserted into several areas of the right cerebral hemisphere in the hope of locating an abscess, a cyst, or a tumor, but without success. The patient died suddenly the next day.

*Autopsy:* Six hours postmortem. External examination revealed nothing save the surgical wound, an old, healed right rectus scar and the evidence of recent tooth extractions. On opening the peritoneal cavity, dense fibrous adhesions were found at the site of a former appendectomy. The heart and aorta showed nothing but a moderate atheromatous degeneration of the latter. The spleen, liver, gastro-enteric tract, pancreas and genito-urinary tract were all essentially normal. The lungs weighed 530 gm. right, and 350 gm. left. Scattered throughout all their lobes were small, irregularly shaped, lobulated nodules of firm, grayish white tissue (about twelve in all) and varying from 2 to 20 mm. in diameter (Fig. 1). These were situated just beneath the pleura and one of them was at the center of a dark red, wedge-shaped area of infarction. Although the large pulmonary vessels were free from emboli, the smaller ones near some of the subpleural nodules were plugged with dry, grayish red material resembling ante mortem clot. The lung tissue not involved in the lesions just described was crepitant and dry. The hilic lymph nodes were not remarkable excepting for one, in which there was some grayish white tissue closely resembling the subpleural nodules and which was slightly larger than its fellows.

When the calvarium was removed very little blood clot was found at the operative site, the meninges were tense and there was marked flattening of the convolutions, particularly on the right. Horizontal sections revealed two large nodules of abnormal tissue in the right cerebral hemisphere (Fig. 2). The larger measured 3 by 2.5 cm. and was situated in the anterosuperior portion of the temporal lobe just posterior to the Sylvian fissure. It was well demarcated from the surrounding brain tissue and could be easily shelled out of its bed, except at one point anteriorly, where it was in contact with, and apparently attached to the meninges of the Sylvian fissure. The cortex could be seen to be pushed aside and to have undergone pressure atrophy in the neighborhood of the nodule. At the center of the mass was considerable hemorrhage and softening. The smaller nodule was spherical and measured 2 cm. in diameter; it was situated in the anterior central gyrus, lying between the fissure of Rolando and the precentral sulcus. It was in contact with the meninges ex-

three sorts: coarse collagen fibers, fine reticulum fibers, and fibrils that were finer, usually blacker and straighter than these. All three types were intimately intermingled. The third group seemed to branch and arborize about the cells in the tumor nests. They were more numerous in the pulmonary metastases than in the meningeal tumors, although present in both situations.

In order to ascertain if possible whether these were nerve endings or not, the following stains and impregnations were used: to rule out fibroglia, the Mallory phosphotungstic acid hematoxylin stain; to rule out elastic tissue, Verhoeff's stain, and to rule out reticulum, the Bielschowsky-Maresch silver impregnation as modified by Foot.<sup>10, 11</sup> This was used in its two variants: the slow one with a preliminary 48 hour impregnation with 2 per cent silver nitrate followed by impregnation with silver diammino hydroxid, and the rapid one without the preliminary treatment with silver nitrate and the substitution of silver diammino carbonate for the hydroxid. This method is essentially the same as that used in the frozen sections, but differs in being applied to paraffin sections. The pyridin treatment has no particular effect excepting to cut down the chances of precipitates.

None of these methods demonstrated the fibrils seen in the frozen sections. There were occasional fibroglia fibrils in the fibroblasts, beautiful neuroglia fibrils in the brain cortex, with phosphotungstic acid hematoxylin; Verhoeff's method demonstrated elastic tissue fibers in the alveolar walls of the lung and in the vascular walls (a modification of this stain failed to demonstrate myelinated fibrils in the masses of tumor cells, although it worked perfectly in the brain tissue) and the silver impregnation of paraffin sections brought out copious collagenous and reticular fibrils in the stroma of the tumor, but no "nerve endings."

As this apparently strengthened our assumption that we had found such fibrils in the frozen sections, a method was used that employs precisely the same solutions as the one with which we had such success, but differs from it in substituting bromuration for the pyridin bath. This method had been found excellent for neuroglia cells and fibers. The bromuration was accomplished by a modified Globus technique:<sup>7</sup> the sections were left in 4 per cent ammonia for 24 hours, placed in 10 per cent hydrobromic acid for 8 or more hours and transferred to Cajal's fixative (15 per cent formalin, 6 per

Masson had purposely somewhat neglected the silver impregnations and that a method derived from Cajal and Bielschowsky traditions by Foot<sup>8</sup> had always demonstrated nerve fibrils very well, we subjected frozen sections of the tumor to this treatment and obtained the striking results shown in our illustrations. The method is as follows:

*Silver Impregnation of Tumor Fibrils:* Fixation in 10 per cent neutral formalin, frozen sections at 20 microns. Place sections in equal parts of pyridin and absolute alcohol for 30 minutes and transfer to pure pyridin for from 3 to 10 hours. Wash out the pyridin in several changes of water and transfer to distilled water. Impregnate for one-half to one hour in the ammoniacal silver solution (silver diammino hydroxid) devised by Kubie and Davidson,<sup>9</sup> which is prepared as follows:

To 10 cc. of 10.2 per cent silver nitrate add strong ammonia drop by drop until the resulting precipitate is just dissolved. Add 10 cc. of 3.1 per cent sodium hydroxid and dissolve the resulting reprecipitation in just enough strong ammonia, added dropwise, to clear the solution. Do not add more ammonia than is needed. Make up to 100 cc. with distilled water and heat to 45 C.

After the sections have been impregnated in a closed vessel (to prevent precipitate which results in open dishes) in the incubator at 37 C, they are washed in 2 changes of distilled water and reduced in a mixture of formalin and sodium carbonate (40 per cent formalin 1 cc., 1 per cent sodium carbonate 3 cc., distilled water to make up 100 cc.). Two minutes or more will be sufficient. The yellow and gray sections are then washed in tap water and toned for 2 or more minutes in 1:500 gold chloride, aqueous. They are passed through tap water into 5 per cent aqueous sodium thiosulphate for 2 or more minutes and then washed in water and mounted in balsam in the usual manner. Mount sections on slides from water and treat with the alcohols, flood with thin celloidin, wash in absolute alcohol, oil of Origanum (to clear) and xylol. This avoids wrinkling and tearing, and is the usual Mallory and Wright technique for mounting frozen sections. After covering the sections with celloidin, a counterstain of Van Gieson's picric acid-acid fuchsin, or of aqueous eosin may be used if desired.

*Results of Silver Impregnation:* With this method a complicated network of fibrils was brought out, as shown in Fig. 5. These were of



## SUMMARY

Two cases of melanoma, presumably primary in the meninges, are described: one of them showed a small primary tumor in the choroid plexus with a copious petechial metastasis throughout the meninges of the brain and cord; the other was striking because of the presence of two good sized tumors in the meninges, with metastasis to the lungs, a very unusual event in the case of cerebral tumors.

It is believed that the facts brought out in the study of these cases point very strongly to the validity of Masson's argument as to the nervous origin of melanoma; these tumors had nothing to do with the skin, so far as we could ascertain, and they showed the presence of fibrils that could only with difficulty be interpreted as representing anything else but fibrils in some way connected with peripheral nerves.

cent ammonium bromid) for several hours, and then impregnated by the method already given for frozen sections. The result of this was striking: the fibrils were not only completely suppressed, but the reticulum impregnation was definitely enhanced; no fibrils were found running between the cells as before, while the reticulum network that forms the stroma was brought out in its most minute detail, as shown in Fig. 6. The reticulum formed baskets about the aberrant cells, but the appearance of these and the "nerve fibers" was totally different, morphologically speaking. Furthermore, this technique demonstrated melanoblasts where they had not been evident with other methods. Their melanin granules were impregnated a deep black when "ripe," brownish in the more immature cells and, in such cases, situated about the periphery of the cell in a half-moon. This method, then, affords a specific means of identifying melanin, and demonstrating melanoblasts and immature melanoblasts in tumors.

Further to check up on the nervous character of the fibers in question, sections of quiescent nevi were made and fibers quite similar to these readily demonstrated in the cell nests. They were, however, more varicose, better differentiated and thicker than those in the malignant tumors, and they were more similar to those seen in the Wagner-Meissner corpuscle. Two points remained to be cleared up: were the pictures in our cases similar to those in melanomas arising in the skin, and were the "nerve endings" demonstrable in other types of tumor? To clear the first we made sections from a melanoma of the skin over the buttock and of metastases from another case, primary in the skin, in lymph nodes. In both cases pictures quite similar, if not identical with those in our meningeal cases, were obtained. To answer the second point, sections were made from an epidermoid carcinoma of the scalp and from a fibrosarcoma of the breast. In the former, no fibrils suggesting nervous origin were demonstrable; in the latter many fine reticulum fibrils were seen, but they were totally different in their morphology and did not stain as deeply; furthermore, they were much shorter, unbranched and curly. Sections from a pigmented wart and from a small vascular nevus of the skin failed to present any fibers like the ones in question. It is not our intention to go into the matter any more closely in this paper, the subject is being reserved for further discussion in a subsequent article, after more intensive study.

## DESCRIPTION OF PLATES

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NOTE. Photographs and photomicrographs taken by Mr. Joseph B. Homan of our Department of Medical Art, with the assistance of the authors. Low power photomicrographs about 200, high power about 300 diameters.

### PLATE 132

FIG. 1. Photograph of a section of the lung from Case 2, showing the tumor metastases in two locations as white areas near the periphery.

FIG. 2. Photograph of two sections of the brain from Case 2, showing the large tumor with a dark, hemorrhagic core in the temporal region of the right hemisphere, and the smaller, less necrotic tumor that was situated higher and further forward in the same hemisphere.

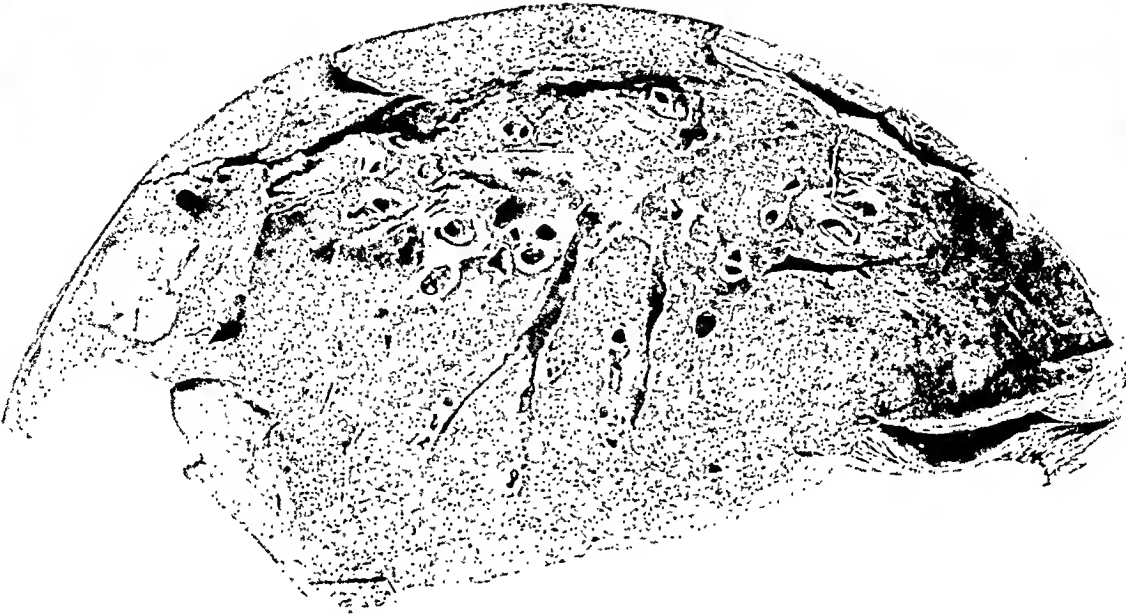
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PLATE 133

FIG. 3. Low power view of silver impregnation of the smaller meningeal tumor.

FIG. 4. The same, following preliminary bromuration. Note the black-stained melanoblasts that were not demonstrable in this tumor by the ordinary, routine stains.



1

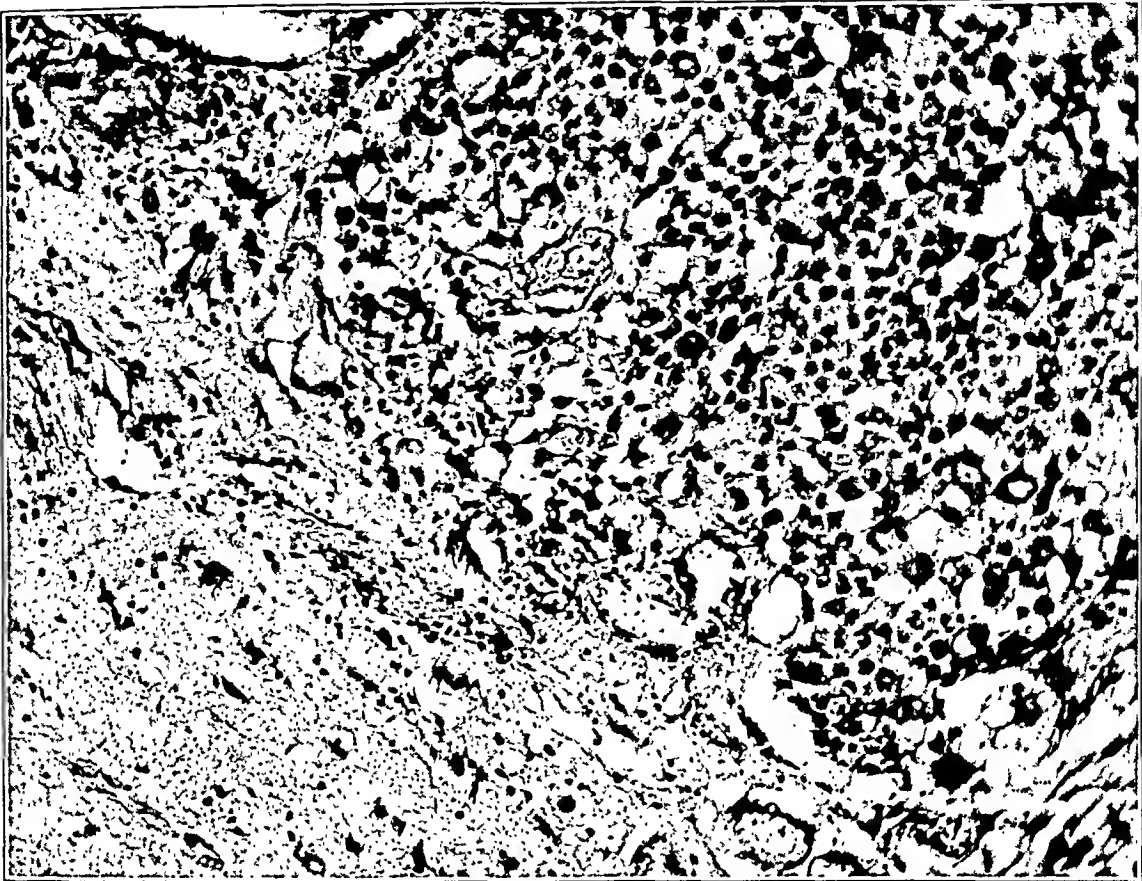


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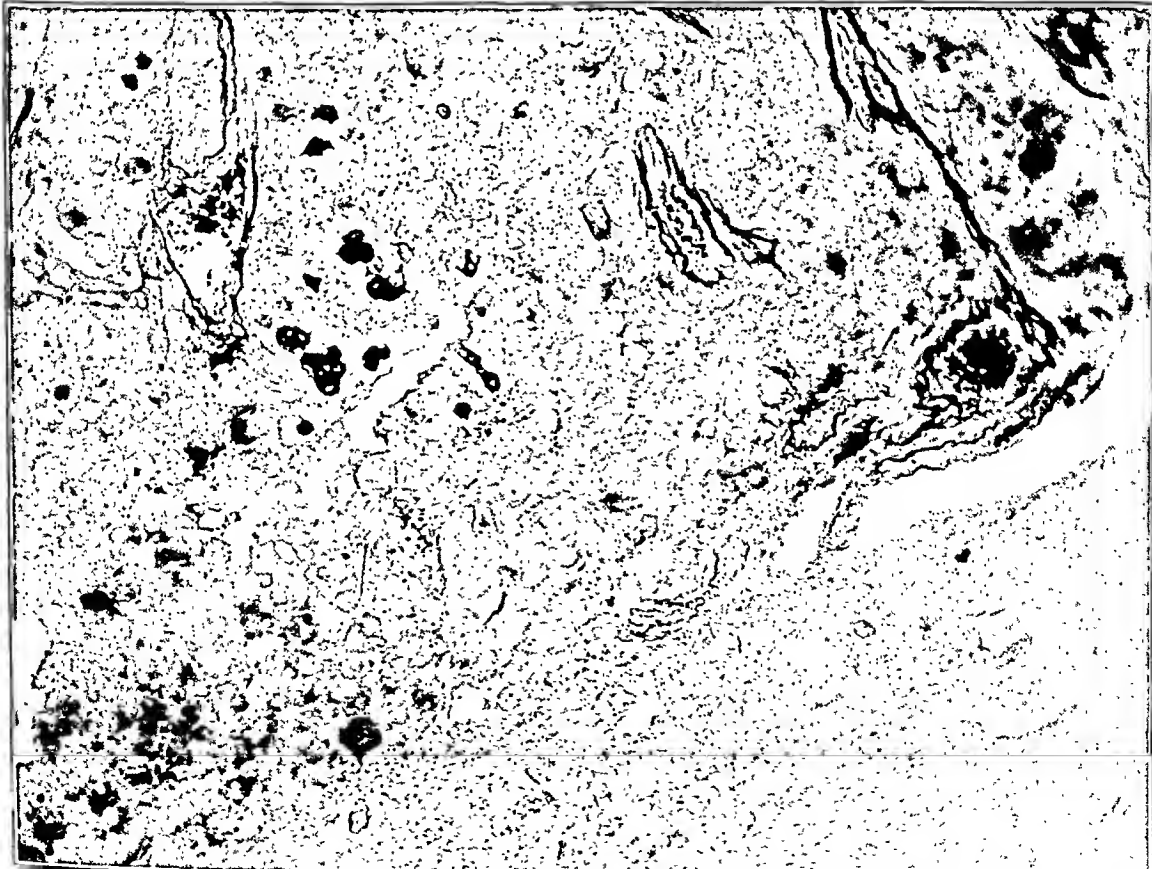
PLATE 134

FIG. 5. High power field from lung metastasis impregnated with silver. Here the varicose fibrils are well shown. Note the paler reticulum and collagen fibers in the background, also the roughly triangular shape of the varicosities.

FIG. 6. High power field, similar to the preceding, impregnated by the same method, but the impregnation preceded by bromuration, which has brought out the reticulum and suppressed the tumor fibrils and cellular detail.

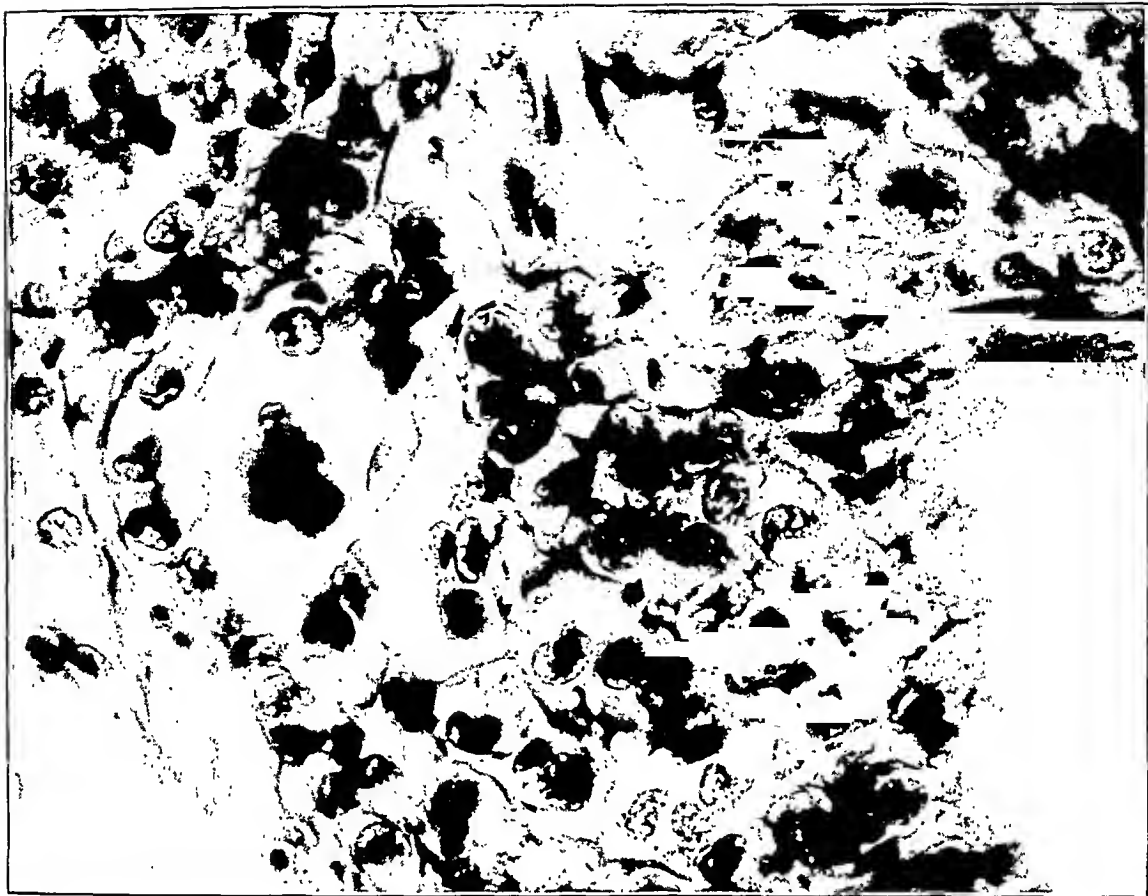


3

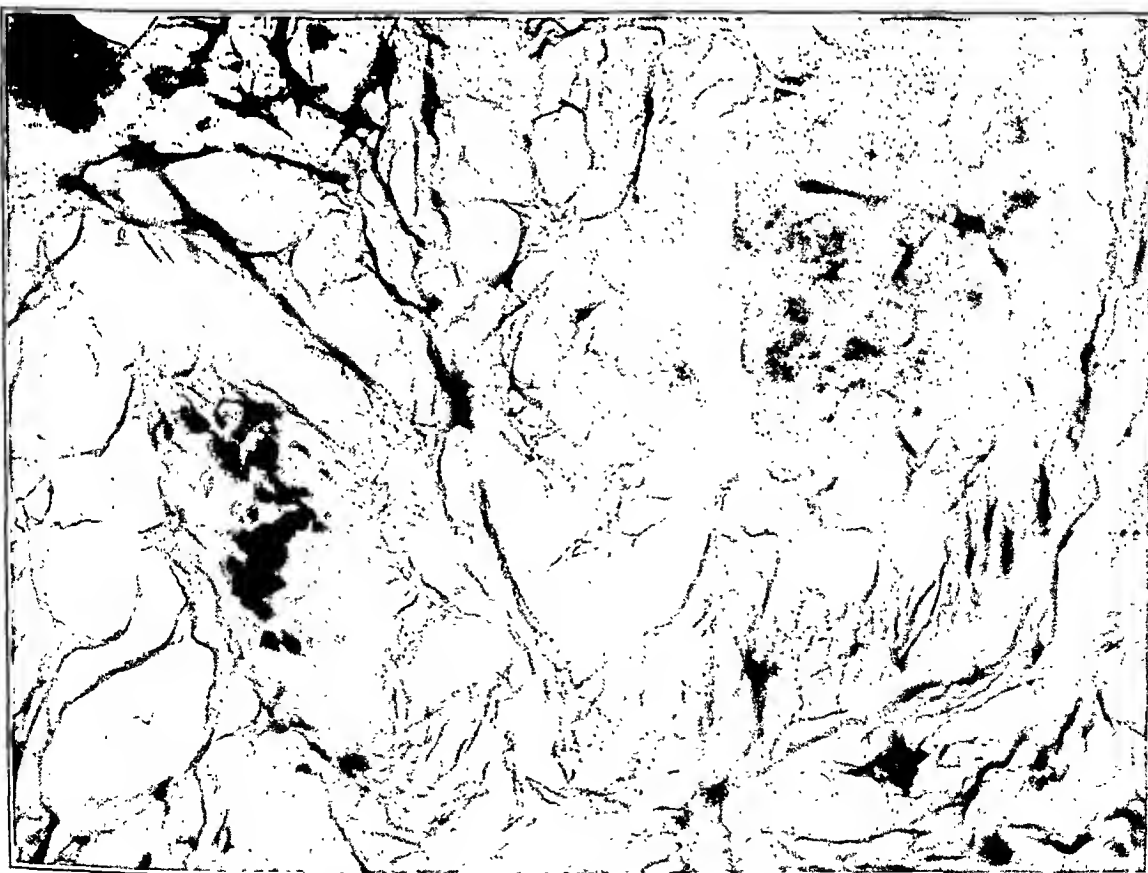








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plausible is this theory that Ewing<sup>3</sup> has espoused it in his textbook on neoplastic diseases, and in other writings.<sup>4</sup> All that remains to be done, apparently, is to improve on the silver impregnations that Masson employed and which he considered rather unsatisfactory. He frankly leaves the use of that technique to others, more skilled in the art of silver impregnation. While making no claim to be qualified for this task, it has occurred to me to employ a method that seemed promising and which has served well in demonstrating nerve fibers in frozen sections of brain. The earlier results of its use are set forth in another paper (Foot and Zeek<sup>5</sup>) in which it is shown to be of distinct value in connection with the demonstration of nerve fibrils in two melanomas of the meninges. It is not the purpose of this paper to expound all the theories concerning the origin of pigmented tumors; the intention is to describe the results obtained in an investigation of these by means of two modifications of a simple silver technique, focussing attention upon the theory of their nervous origin. Other histogenetic theories will thus be rejected by inference.

### TECHNIQUE

*Fixation:* The tumor tissue should be fixed for several days in 10 per cent neutral formalin, cut to 15 microns or more on the freezing microtome and the sections kept for another day in 10 per cent neutral formalin to ensure perfect fixation.

*Impregnating Fluid:* The impregnation depends upon the classical Bielschowsky procedure, modified so as to render the solutions that are used equimolar. The advantages of this modification have been fully discussed in a previous paper (Foot<sup>6</sup>) in which the findings of Kubie and Davidson<sup>7</sup> in this connection were tested out. The impregnating fluid is made as follows: 5 cc. of a 10.2 per cent aqueous silver nitrate solution are poured into a graduate, strong ammonia is added drop by drop until the resulting precipitate is just dissolved, and 5 cc. of 3.1 per cent aqueous sodium hydroxid solution is then added. The precipitate re-forms and must once more be just dissolved in a few drops of strong ammonia, but no more should be added after the solution clears. In this way about 12 cc. of silver diammino hydroxid is produced. It is then diluted to 50 cc. with distilled water that has been heated to about 50 C. It need not be exactly that temperature; the bath should be somewhat hotter than 37 C when the sections are placed in it.

# ON THE SILVER IMPREGNATION OF MELANOTIC TUMORS\*

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## INTRODUCTION

That the pigmented nevi of the skin are, in reality, of nervous rather than of dermal origin and closely related to the neurofibromas, was the theme of an article by Soldan<sup>1</sup> written in 1899. He presented his theory more or less in outline and illustrated it by low power microscopic drawings that were necessarily sketchy, for which reasons one is impressed with the fact that his was a somewhat incomplete presentation of an ingenious idea, more suggestive than conclusive. The technique he employed did not permit its being more than this. Twenty-seven years later, Masson<sup>2</sup> published two scholarly articles on the same subject and came to essentially the same conclusions, but he followed through his line of argument much more carefully and convincingly than did his predecessor, whose work came to his notice only after he had finished his own work quite independently. According to his views, the nevi are really "neuronaevi, nervous tumors" and represent the lawless overgrowth of the cells associated with the terminal sensory nerves, as well as the proliferation of the nerves themselves. As the type cell he takes the "Merkel-Ranvier," or "naevus" cell, which may occur singly in the epidermis, clustered in small groups just beneath it, or arranged so as to constitute specialized tactile organs (Meissner corpuscles). Furthermore, he points out that in the case of nevi these cells are distributed along the trunks and branches of the peripheral sensory nerves which serve as a scaffold for the tumor, lying at first in columnar groups about the fascicles, then in nevus nests that resemble distorted Meissner corpuscles; and finally in small aggregations, or scattered singly, these cells constitute a definite adnexa to the peripheral nerve tree. Masson considers the melanoblasts, or Langerhans cells, to be specialized members of the same cell race, cells specializing in the formation of pigment, rather than serving the purposes of their more numerous, non-pigmented congeners. So well presented and

\* Received for publication July 3, 1931.

ening of the nerve fibrils, making them brown instead. Two minutes or so is sufficient to develop the sections completely.

*Toning:* After a wash in tap water, the sections are toned for 2 minutes in a 1:500 aqueous gold chlorid solution which may be used repeatedly until grayish, monotone sections warn one that it is becoming exhausted. Merck's acid brown gold chlorid in 1 per cent aqueous stock solution has been our standard. The sections turn from brown, yellow and black to very deep violet in the toning bath.

*Fixing:* Sections are washed in tap water and fixed for 2 or more minutes in 5 per cent aqueous sodium thiosulphate, where they become pliable and take on a lighter, more varied color.

*Mounting:* It has been found best to mount the sections out of a large porcelain evaporating dish of water on clean slides, blot them dry with several thicknesses of filter paper and proceed as in the Mallory and Wright celloidin technique. Flood the slide with 95 per cent alcohol from a dropping-bottle, follow this with absolute alcohol, blot, pour on a few drops of very thin celloidin dissolved in ether and a little absolute alcohol, flood once more with absolute alcohol and blot dry with filter paper. The celloidin is cleared by flooding the slide with oil of Origanum and washing in two changes of xylol, after which a drop of Canada balsam and a coverslip complete the process. The celloidin ensures flat mounts and keeps the sections on the slides if counterstains of 5 per cent aqueous eosin, or Van Gieson's stain are desired and employed.

*Remarks:* Fixation should be thorough, as indicated, otherwise grayish, monotone effects will be produced. Glass needles are essential to prevent metal from coming in contact with the silver solution and when rather stout and rounded at the tip, they also prevent tearing of the sections.

## RESULTS

*Pyridin Method:* The keratinized epithelium is very dense and dark brown, the prickle cells of the epidermis and the cells of the mucosa slate blue, the basal cells brown, and the melanoblasts dark brown to black. The nuclei of most of the cells are brown. Collagen fibers are reddish, violet-red, to pink. Reticulum is somewhat darker, brownish to black. Neuroglia cells and fibers are grayish, rather poorly shown. Nerve fibers and terminal fibrils impregnate

*Pretreatment of Sections:*

(a) *Pyridin Method:* If one desires to impregnate terminal nerve fibrils and end organs, it is well to put the sections into a mixture of equal parts of pyridin and absolute alcohol for 15 to 30 minutes; they are then removed with a glass needle, touched to filter paper to remove excess fluid and transferred to a mixture of pyridin 75 parts, and glycerol 25 parts, where they remain overnight. The function of this bath is to prevent precipitates, to restrain the density of the general impregnation and thus to render more precise that of the nerve fibrils. The addition of glycerol has been found empirically to favor a more colorful metachromatic impregnation. Although the bath is not essential, its use is to be highly recommended.

(b) *Bromuration:* A modification of the Globus bromuration of formalin-fixed tissue may be employed to suppress the impregnation of the nervous elements, to accentuate that of the reticulum and to bring out neuroglial tissue when it is present, as in the case of brain tumors. Frozen sections are removed from formalin, soaked in 4 per cent ammonia for 24 hours (after washing off the fixative), and then transferred directly to 10 per cent hydrobromic acid where they remain overnight in an incubator at 37 C. They are then transferred to Cajal's fixative, where it is imperative that they should remain for at least 8 hours. This is composed of 40 per cent neutral formalin 15 cc., ammonium bromid 6 gm., and water to make 100 cc.

*Impregnating:* This is the same in the case of either pretreatment: the sections are washed in two changes of tap water and brought into distilled water, then placed in the impregnating fluid for one hour at 37 C in a tightly stoppered vessel. If the vessel be open to the air, troublesome precipitates of metallic silver will result. In this bath the sections turn dark brown, lighter if they have been bromurated.

*Reduction:* Sections are then well washed in two changes of distilled water and placed in a developer made up as follows: 40 per cent neutral formalin 1 cc., 1 per cent aqueous sodium carbonate (made up with distilled water) 3 cc., and distilled water to make 100 cc. This gives us a 1 per cent formalin reducer that will ensure the maximum contrast and a buffer of 0.0003 per cent sodium carbonate that will afford the most complete reduction of the silver, probably owing to its preventing the formation of formic acid. The use of a stronger buffer is to be discouraged, as it prevents the black-

the sections have been bromurated. As further indication that nerve filaments are impregnated in the pyridin method one finds the terminal organs on the muscle fibers of the tongue, as well as other delicate nerve endings elsewhere.

That all of these are, indeed, nerve, or nerve sheath filaments and not simply connective tissue elements is also indicated by the fact that they are either undemonstrable in bromurated sections, or merely visible as faint shadows. Exceptions are the delicate fringe-like fibrils that border and run into the basal cells of the mucosa and epidermis, and the muscle sheaths, both of which impregnate deeply in the case of either method. The fringe fibrils stop abruptly at the basal layer in the case of bromurated sections, while they continue on into and among the basal cells in that of pyridin-silver specimens. One must bear in mind the very intimate relationship of nerve fibers and the reticulum that supports them, so that it is not strange to find them intermingled in the same structures. The Meissner corpuscles, for instance, show a reticular envelope and transverse trabeculae in bromurated sections, but the nerve filaments and the tactile cells are reduced to mere shadows, so that one seems to be looking at an empty hull.

### PIGMENTED NEVI

A number of nevi of various sorts were collected from the dermatological clinic and the autopsy table and examined by means of the above methods. It is interesting to find how extremely easy it is to collect moles at autopsy, as well as to observe how varied is their nature. The term "nevus" is admittedly very loose and several tumors or congenital defects are classified under this name. We are not interested in the vascular nevi, or the pigmented warts (Fig. 4), or papillomas, in this paper, excepting as they show a complete absence of "nevus nests" and the filaments that characterize these.

The pigmented, melanotic or neuronevi may be divided into two main subclasses, the superficial and the deep, as Masson has indicated. Various subheadings of these two divisions could readily be devised according to the presence or absence of pigment, the papillary or non-papillary character of the epidermis, and so on. The situation of the tumor, whether deep or superficial, seems to determine real differences in its architecture and may therefore be

dark brown to black. Muscle fibers are gray to brown, their sarcolemma black.

*Bromuration Method:* Keratinized epithelium brown, prickle cell layer and mucosa pale gray and stippled, basal layer slightly darker gray, melanoblasts prominent and black. The nuclei are poorly, if at all, impregnated. Collagen fibers and nerves are pinkish to violet. Reticulum is sharply black. Neuroglia cells and fibers are blackish, or dark brown. Muscle fibers are grayish, sarcolemma sharply black.

It will be seen from this that the two methods are roughly complementary; in the one the nerves are brought out, in the other the reticulum and neuroglia. It cannot be said that either method completely suppresses elements noted in the other. As they are present in both cases they will be visible, but they will tend to stand out or to retire from prominence in one or the other instance, and therefore be more readily recognized. The important thing is that the pyridin method brings out the terminal nerve fibers and their end organs, while the bromuration method suppresses these to a minimum of visibility and emphasizes the connective tissue elements at their expense, or the neuroglial, if brain tissue be in question.

### CONTROL OBSERVATIONS ON NORMAL TISSUE

Skin from the ball of the great toe and mucosa from the side and tip of the tongue were examined by both methods. The pyridin procedure demonstrated Meissner corpuscles, end bulbs in the sub-epithelial tissue and non-medullated nerve fascicles. The medullated nerve trunks were also readily recognizable. Examples of these are shown in Figs. 1, 2 and 3. One should distinguish carefully between the arrangement of nerve fibrils in the three types of structure: in the Meissner corpuscles they run in a fine network with transverse filaments connecting the peripheral, encircling ones and coursing between the transversely arranged tactile cells; in the end bulbs the fibrils are coarser and often zig-zag back and forth with triangular varicosities at the angle of their reflexion and their tactile cells are less prominent; in the fascicles the naked nerve fibers run parallel for a short distance after emerging from their myelin sheaths in the nerve trunks, to become a confused skein or tangle surrounded by a connective tissue envelope. In any case, reticulum is intimately intermingled with the nervous elements, but is not prominent unless



a tendency on the part of the nevus cells to differentiate into melanoblasts, or Langerhans cells (Fig. 7), in which case the innervation of the nevus nests was much less complex and striking than in the more usual variety. The fibrils tended to be much coarser in the melanoblastic type. The factor of pigmented nevus cells has strikingly little effect upon the gross appearance of the mole, for some very brown moles were found to have the pigment concentrated in the basal layer of the epidermis, while the melanoblasts in the nevus nests were very few in number. An equally brown mole might show the reverse picture microscopically, with numerous melanoblasts in the nevus nests and no particular increase in the epidermal pigment.

*Deep Neuronevi:* These showed the nevus nests to be more deeply situated in the derma and to have an affinity for the deep hair follicles, alongside of which they were apt to be concentrated (Fig. 8). The nests showed a closer resemblance to end bulbs, or to the tangled nerve fascicles than they did to Meissner corpuscles. This is natural when one takes into consideration their situation, which is closer to those structures than to the more terminally situated tactile bodies (Fig. 9). In this case, too, there was marked proliferation of connective tissue elements about the nerve fibers and nevus nests and the similarity to neurofibroma was even more striking. Unfortunately, this type of tumor was in the great minority of those collected, so that it could not be studied as intensively.

In all cases, bromuration brought out the connective tissue elements at the expense of the nervous, just as in the controls on normal tissue, and indicated that the latter were nerve and not reticulum fibrils.

### MALIGNANT NEURONEVUS — MELANOBLASTOMA

Logically following the classification, we come to the malignant members of the group, the melanoblastomas, so-called "melanosarcoma" and "melanocarcinoma." It seems futile to attempt a rigorous classification of these, since they are completely lawless and defy classification. A number of examples were collected; malignant melanoma of the skin, two cases originating in the meninges, a metastasis from a dermal melanoblastoma to the axillary nodes, and so on. They all conformed to one loose law, however, in that they resembled the benign forms in their general arrangement and in the fact that they invariably possessed the elaborate neurofibrils of

useful in classification. Even so, any attempt at strict subclassification appears to be rather futile on account of the essentially close relationship of the various tumor elements to each other.

*Superficial Neuronevi:* A study of these showed Masson's description to be exceedingly accurate. All that remains to be added to it is the fact that the distribution of nerve filaments among the nevus cells is even more elaborate and copious than he has indicated. It was found that large nerve trunks entered the base of the tumors, branched and in turn gave off the non-medullated fascicles stressed by Masson. These then appeared to break up into broad, reteform sheets that became subdivided into naked fibers that branched and finally terminated in very delicate fibrillae that arborized about the cells of the nevus nests. The nests were found to be distributed as Masson has so well described: at first elongated and columnar about nerve fascicles, later spheroidal and more closely resembling distorted Meissner corpuscles. In fact, one could pick out isolated instances where the resemblance was so striking as to give rise to doubt as to whether one were not observing a normal corpuscle rather than a nevus nest. The nests extended up into the dermal papillae and were not infrequently covered by a comparatively thin layer of epidermis. There was never any question as to distinguishing between epidermal and nevus cells — with the silver impregnation their appearance is totally dissimilar. The epidermal cells were quite free from fibrils, rather granular, decidedly denser than the nevus cells which were pale, possessed of vesicular nuclei and usually surrounded by the arborizing nerve endings, as seen in Figs. 5 and 6.

Most of the moles collected showed slight variations of this picture. In some there was scarcely any melanin, excepting for a variable amount in the basal layer of the epidermis; in others this was very considerable and there was "spilling over" into the melanoblasts of the nevus nests near the epidermis. The nerve filaments, too, varied from extremely delicate tendrils to rather stout fibrils; one tumor would show one variety (particularly a pedunculate "white mole"), while another would show coarser fibrils with less tendency to elaborate arborization among the nevus cells. There was also marked variation in the amount of collagen and reticulum in the specimens collected, but most of them showed an almost fibromatous proliferation of collagen fibers about the nerve trunks and fascicles near the base of the tumor. One or two tumors showed

Some of them, as has been remarked, show a tendency toward the production of melanoblasts. It therefore seems obvious that any, or all of the cells of the sensory nervous adnexa might be considered as type cells of the same cell race ("*de la même souche cellulaire*," as Masson puts it); even the sheath cells of Schwann, which have long been considered by embryologists as neuroglial, in contradistinction to the mesoblastic cells of the adnexa, must be considered as potential participants in the process. The variegated appearance of the malignant types of the tumor would strengthen the assumption of racial similarity between the cells of the adnexa. One might, then, speak of "neurofibroma" developing up to the point where the myelin sheath ends in the nerve trunks and of "neuronevus" when the tumor is situated beyond that point, including the end organs of the nerve.

Criticism of this theory may be foreseen and, one hopes, partly forestalled. A criticism directed toward the question of the nervous character of the end fibrils under discussion has been answered to the best of my ability in this paper. Another criticism, already encountered in personal discussion, centers about the point as to the production of these fibrils — do the tumor cells produce them? Obviously not, for nerve fibers are the prolongations of the axones of nerve cells and as these fibrils are found in the benign and malignant nevi, and in the metastases of the latter as well, they must be accounted for. This can best be done on the basis of "innervation." Is it not probable that the proliferation of the cells of the adnexa provoke a proliferation of the nerve, or nerve sheath fibrils from their parent stalk, as it were? Do not nerves grow out in just this manner into the various embryonal primordia and in regenerating nerve stumps, and could this not be a similar process of growth?

Another criticism might rest upon the absence of such tumors, as a rule, from sites liberally supplied with Meissner corpuscles — the tongue, the balls of the fingers and toes and the like. This criticism is of merely negative value and lays too much stress on the nervous elements. If Meissner corpuscles, or nerves for that matter, be present in a certain area, such tumors could well arise there, but they need not necessarily do so.

these, possibly less well organized and differentiated. It was also noted that some of them tended to produce the amelanotic cell nests, while others ran to the production of melanoblasts at the expense of nevus cells. They tended to be superficial and to extend well up into the epidermis, but as they showed progressively invasive growth at the base, they also extended more or less deeply into the derma. Some of them exhibited marked connective tissue proliferation; others were of a more adenoid type and tended to ape adenocarcinoma in their arrangement and appearance, particularly in their metastases. The structure of these tumors is too well known to warrant further description here — suffice it to say that their relationship to the benign pigmented nevi, or neuronevi, was strikingly manifest in most instances. Only when they became so anaplastic as to resemble adenocarcinoma did their resemblance lag, and in these cases one could still always find some areas where it was still maintained, particularly between the cell nests where younger aggregations of cells still held to the nevic type of growth. A melanoblastoma is shown in Fig. 10.

### DISCUSSION

In the earlier paper <sup>5</sup> it was shown that the fibers of these tumors were not demonstrable by the methods devised for specifically staining collagen, reticulum, fibroglia, elastic tissue, neuroglia and the like; it was also noted that no such fibrils, presumably of nervous origin, were present in the cell nests of epidermoid carcinoma (which would probably resemble melanoblastoma the most closely) or in fibrosarcoma (which possesses innumerable reticulum fibrils), see Figs. 11 and 12. The bromuration method, as I have repeatedly stressed, strengthens one's belief in this nervous origin.

Masson has covered the theory of the histogenesis of this tumor group very thoroughly, so that it would be presumptive to repeat what he has already said, but one might elaborate on his ideas a little. For example, it is not to be understood that the Meissner corpuscle is necessarily the starting point for the tumor; some nevus nests resemble this organ very strikingly and, in such cases, it may well have been their point of origin; others, however, more nearly resemble end bulbs, still others nerve fascicles and some may show so much fiber proliferation that the nevus cells become obscured.

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## DESCRIPTION OF PLATES

NOTE. All the photomicrographs were taken by Mr. Joseph B. Homan of our Department of Medical Art, assisted by the author. They were made from frozen sections impregnated by the pyridin-silver diammino hydroxid method described in this paper. The low power pictures show about 200, the high power about 800 diameters enlargement. No counterstain was used in any instance. The detail in the photographs, particularly Fig. 10, shows the excellence of the method for general purposes of photomicrography, such as the bringing out of nuclear detail, and the like.

## PLATE 135

FIG. 1. Low power view of a group of Meissner corpuscles in the submucosa of the tip of the normal human tongue. They project upward between the epithelial papillae.

FIG. 2. High power detail of the tip of one of the above corpuscles, from the same field. Note the complexity of the arrangement of the fibrils and their transverse distribution.

## SUMMARY AND CONCLUSIONS

The microscopic examination of material from normal skin and mucosa, a variety of nevi and several melanoblastomas, by means of a thoroughly reliable silver impregnation, seems to bear out Masson's theory as to the nervous character of pigmented moles and their malignant relatives. That the "Merkel-Ranvier," or "nevus" cell, divisible into tactile cells of various sorts, and the cells of the non-medullated nerve fascicles, all represent various phases in the life of one cell type in these tumors seems fairly evident, if not proved. That these cells may take the form of scattered individuals, nests resembling Meissner corpuscles, melanoblasts, or neurofibromatoid complexes seems clear. In the case of the malignant tumors, it is only natural to expect more or less atypical growth and anaplasia and a return to structures representing stages in the fetal development of the cell. Alveolar and gland-like structures seen in "melanocarcinomas" should not, therefore, prove to be a very disturbing note in the theory.

The silver impregnation as a means of recognizing and classifying these tumors cannot be too highly recommended; it is simple in operation and actual experiment proves that it can be successfully carried out at the very first attempt. It is to be regretted that it cannot, as yet, be successfully applied to paraffin sections, but as experiments are now under way, this difficulty may be solved. The striking difference between the epidermal and the nevus cells, when impregnated by this method, is at once evident, the association of fibrils with the latter affording a reliable criterion that is immediately applicable. In closing, it would not be trite to reiterate Masson's warning to investigators along these lines; please do not rely upon old and outworn methods while endeavoring to check up on this theory, but use those that Masson has perfected, or the one herein described.

## PLATE 136

FIG. 3. High power view of a group composed of a nerve fascicle containing medullated and non-medullated fibers, and of an "end bulb," smaller than the fascicle, with a vessel lying between them. Note the fibers entering the bulb at one side. These structures often lie near the smaller vessels of the tongue.

FIG. 4. Low power picture of a pigmented wart, or papilloma. Note that the pigment, impregnated black with silver, is limited to the cells of the basal, epidermal layer. There are no nevus nests to be seen.



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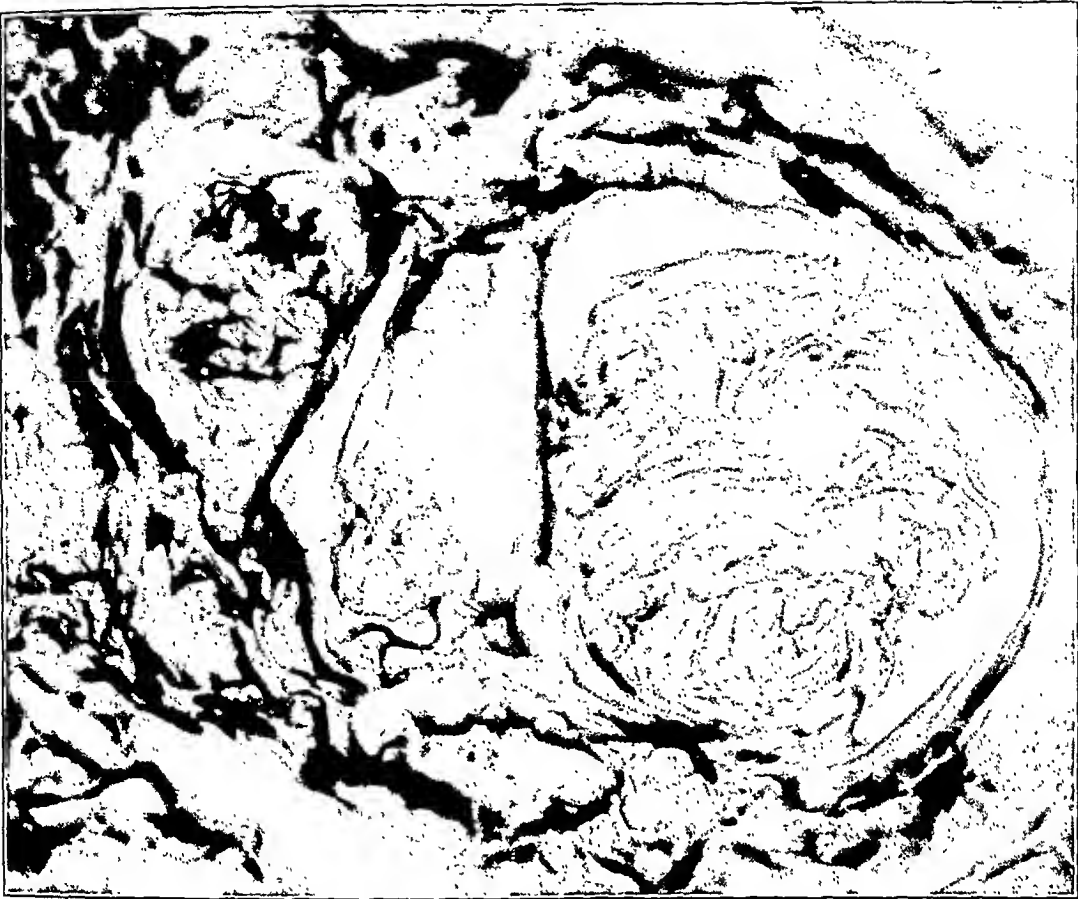
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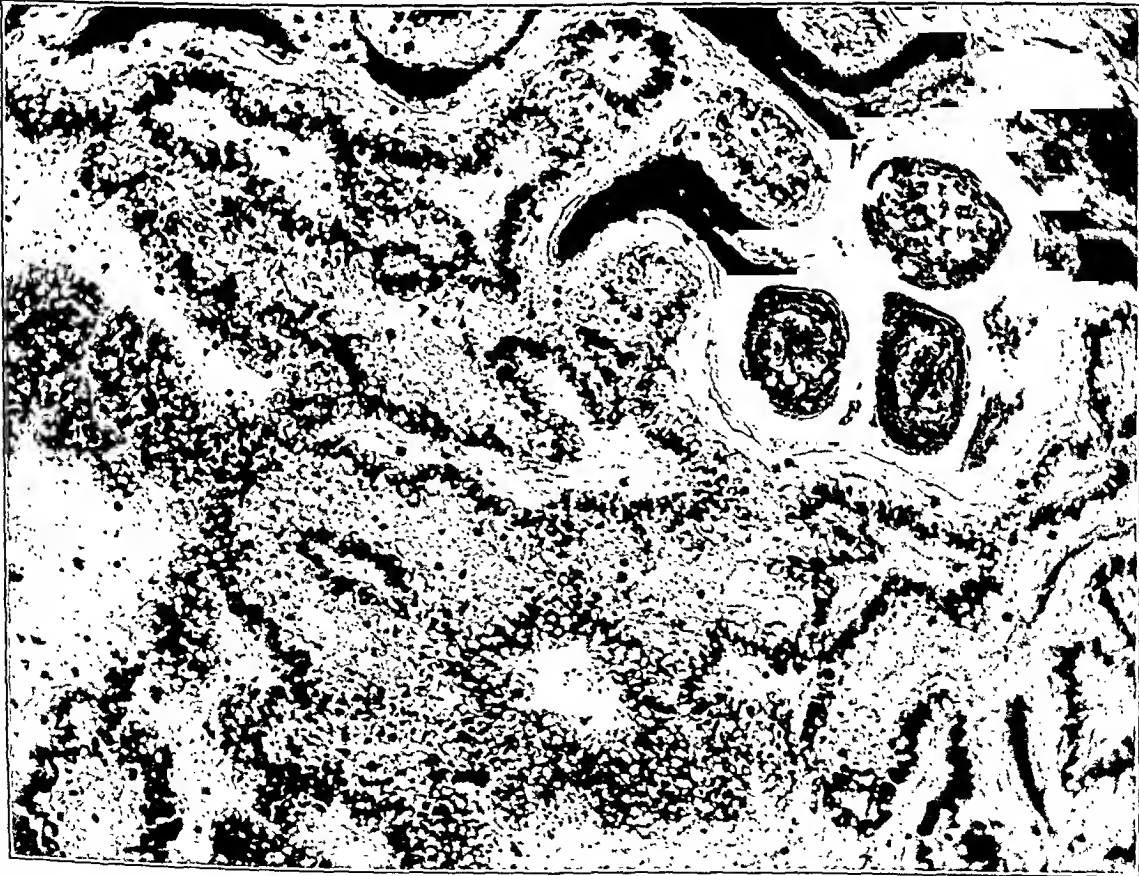
PLATE 137

FIG. 5. Low power field of a non-pigmented mole or "white nevus," to show the nevus nests lying superficially and extending out nearly to the surface. Note the marked difference in the appearance of the nevus, the epidermal cells and the fringe fibers that normally run between derma and epidermis, running between the nevus cells and the epidermis in this case.

FIG. 6. High power view of a superficial, pigmented, non-papillary nevus, to show the complex fibrils that branch and arborize amongst the cells of the nevus nests. Here and there varicosities may be seen on the coarser fibers.



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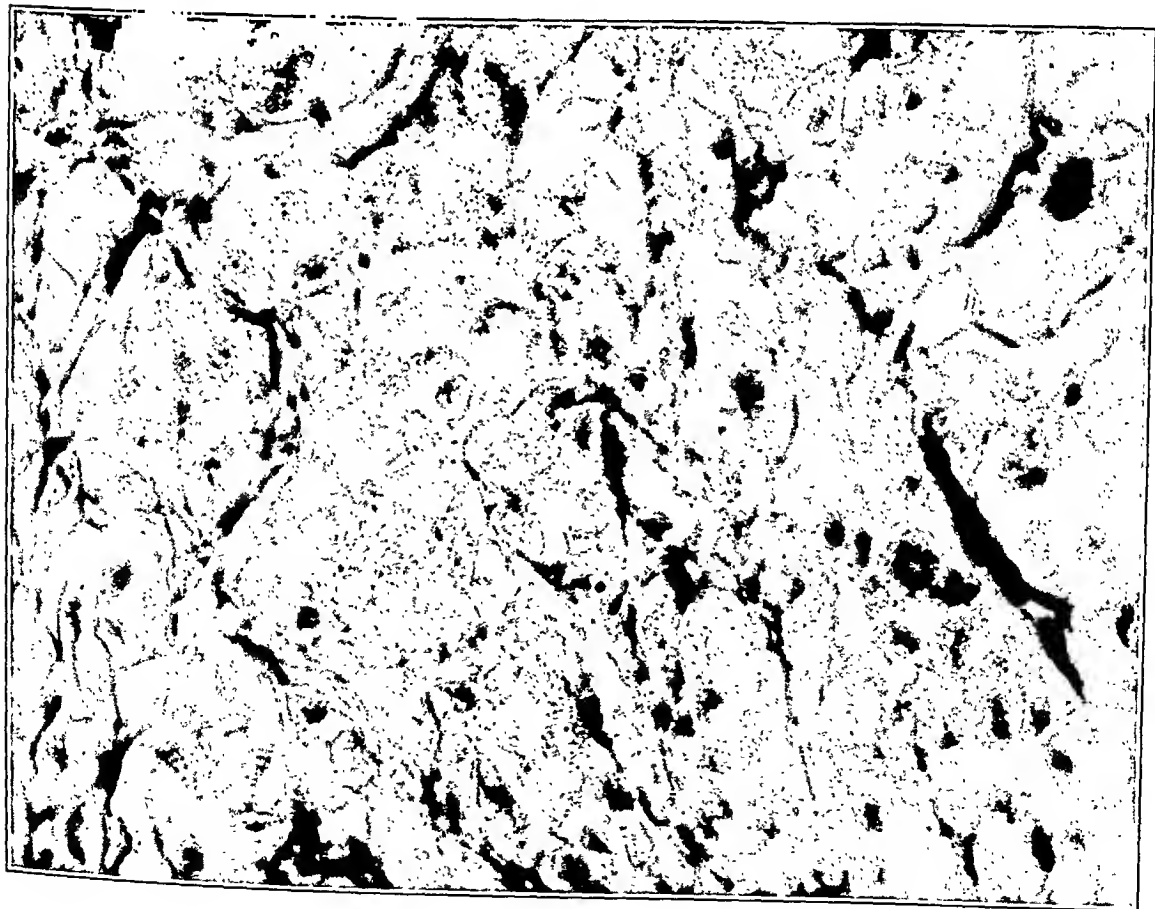
Foot

PLATE 138

- FIG. 7. Low power view of a superficial "melanoblastic" nevus. Note the nests of melanoblasts deeply impregnated with silver and the comparative scarcity of fibrils in these. Other nests in the same tumor were comparable with those in Fig. 6, although their fibrils were much coarser and their cells more sparsely strewn.
- FIG. 8. Low power topographic view of a deep nevus, showing the comparatively unaffected epidermis and "pars papillaris" of the dermis, the tumor lying almost in the subcutaneous tissue. The pigment in this "brown spot" is concentrated in the basal layer of the epidermis. Note the nerve trunk entering the area of nevus nests and branching to form a reteform plexus in which their cells are embedded.



5



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Foot

Silver Impregnation of Melanotic Tumors

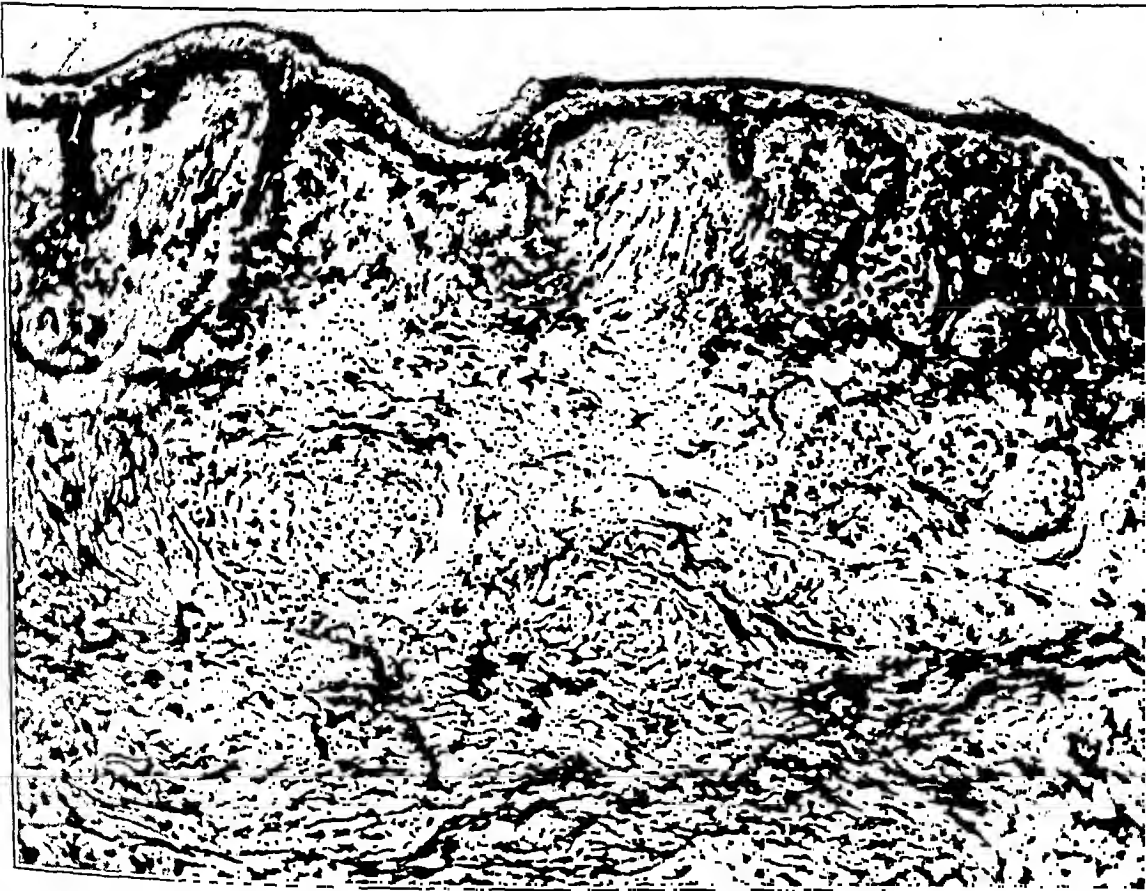
PLATE 130

FIG. 9. High power detail of a nerve in which are embedded a number of nevus nests, taken from the tumor shown in Fig. 8. The black masses of material are silver precipitate, the result of impregnation in an open vessel. The specimen was impregnated during the experimental stage of the investigation and had to be used for illustration as no other deep nevus was collected after the method was perfected.

FIG. 10. High power field from a melanoblastoma of the buttock ("malignant melanoma"), showing very delicate fibrils in all their ramifications.



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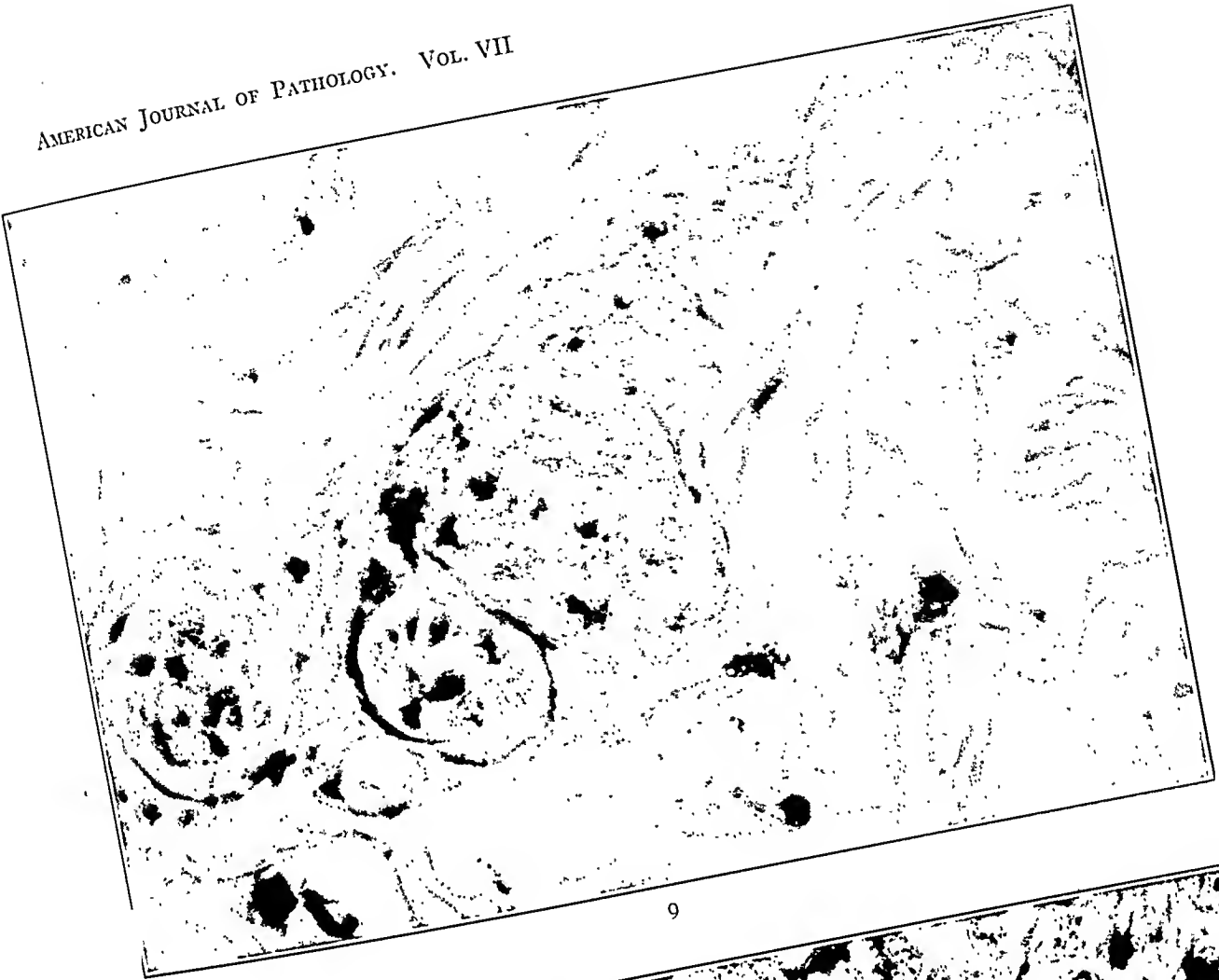
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Silver Impregnation of Melanotic Tumors

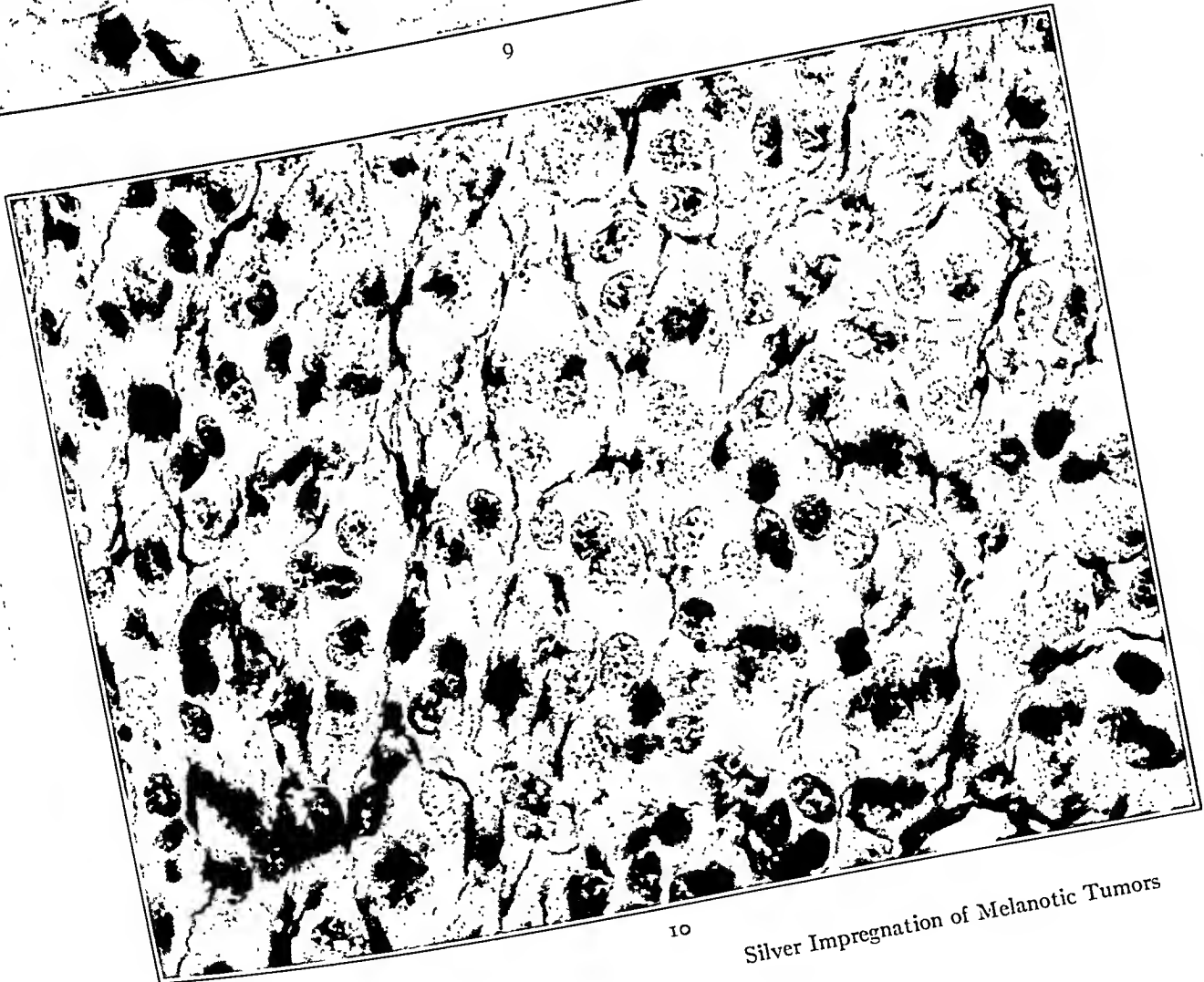
PLATE 140

FIG. 11. Low power view of an epidermoid carcinoma to show the absence of fibrils from the tumor cell nests.

FIG. 12. High power field from a fibrosarcoma of the breast showing the dissimilarity of the fibrils of such a tumor to those of the nevus group. These are reticulum fibrils, for the most part, and do not branch, neither do they terminate in arborizations around the tumor cells as do those of the nevic tumors.



9



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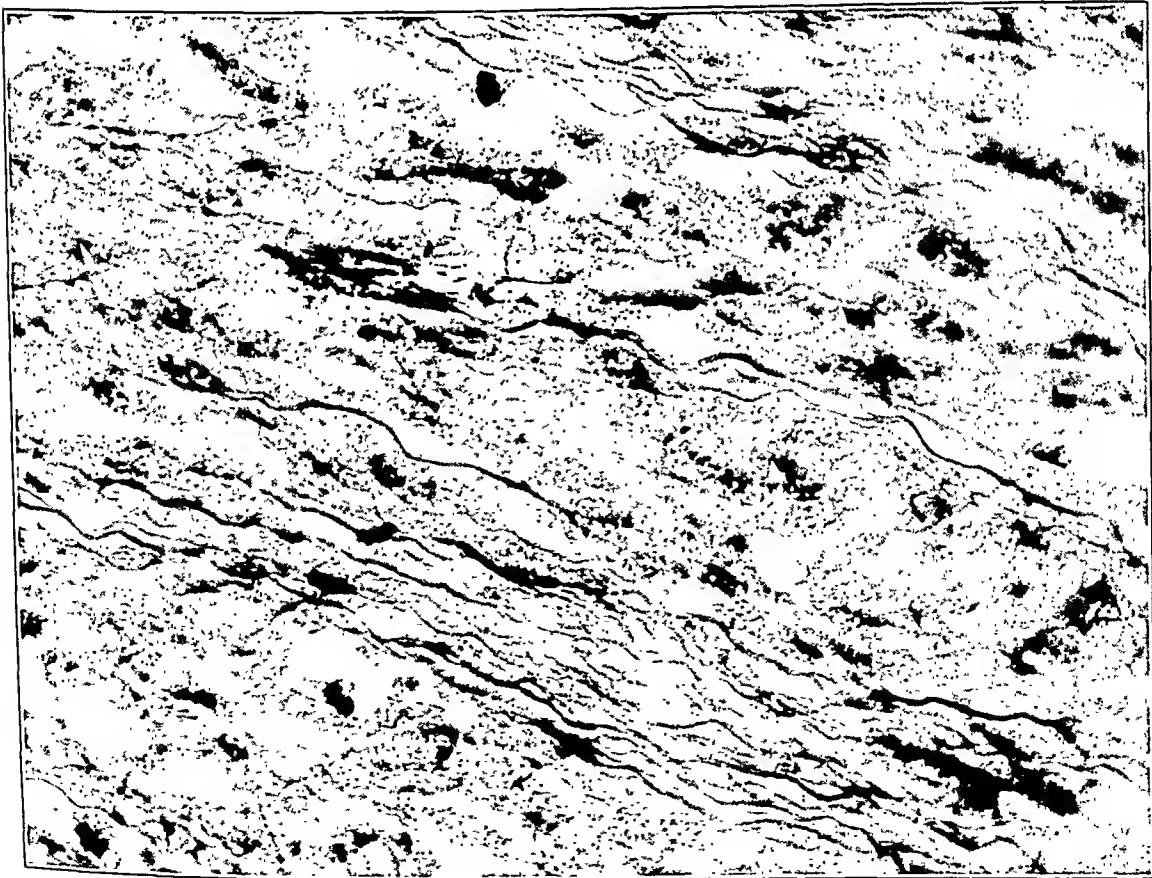
Silver Impregnation of Melanotic Tumors







11



12

the pertinent facts known with regard to unattached retroperitoneal tumors, and this we shall endeavor to substantiate in the following review.

## REVIEW OF EMBRYOLOGY OF THE UROGENITAL APPARATUS

This is intended to be a very brief review of the embryology of the urogenital apparatus and its purpose is merely to orient the reader with regard to facts he may not readily recall.

Structures to be discussed in this résumé are:

1. Pronephros.
2. Urogenital fold.
3. Mesonephros.
4. Metanephros.
5. Genital cell.
6. Genital gland.
7. Müllerian duct.
8. Adrenal cortex.

The last is added because in development it is closely associated with the urogenital apparatus.

The following points will be considered:

1. Origin of the structure.
2. Time of appearance, time of complete development and time of degeneration (if such occurs).
3. Extent of the structure.
4. Participation of the structure in the formation of adult organs.
5. Anatomical relationships of the developing structures.

1. *Pronephros*: The pronephros forms from the primitive segment stalk (cephalic end of the nephrogenic cord) by an evagination of the celomic epithelium into the parietal mesenchyme. In the human the first anlage is in a 1.73 mm. embryo. Complete formation is almost reached in a 2.5 mm. embryo (23 pairs of segments), and degeneration is well advanced in a 4.25 mm. embryo. The approximate stage of complete disappearance is the 4.9 mm. stage. The pronephros extends from the seventh to the fourteenth primitive segments. A pronephric segment consists of a principal tubule, a nephrostome canal and a glomerulus. The segments are connected by a collecting duct (Wolffian duct). The free terminal part of the excretory duct probably forms "in loco" from the mesenchyme. It

## MASSIVE UNATTACHED RETROPERITONEAL TUMORS \*

### AN EXPLANATION OF UNATTACHED RETROPERITONEAL TUMORS BASED ON REMNANTS OF THE EMBRYONIC UROGENITAL APPARATUS

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The occurrence of retroperitoneal neoplasms arising independently of the adult urogenital organs has, to date, eluded scientific explanation. Until about 1880 these tumors were diagnosed as sarcoma or pancreatic cyst, and dismissed without further consideration or study. Howship in 1871 had observed a composite tumor possessing certain characteristics similar to dermoid teratomas commonly found in the ovary. In 1889 Bassini described a cystic adenoma morphologically resembling a pseudomucinous cystadenoma of the ovary, and Frank in 1894 observing a similar tumor attributed its histogenesis to an accessory ovary. Since 1894 instances of unattached retroperitoneal tumors have been reported in the medical literature averaging somewhat less than one case a year. Occasionally, authors of these case reports have added to the diversity of histological features that may be associated with these neoplasms. These now include glomeruli, renal tubules, rete structures, bone, hair, sweat glands, fat, smooth muscle, uterine mucosa, chorion epithelium and so on.

We have been privileged to study seventeen cases of unattached retroperitoneal tumors, and having correlated our findings with the available information obtained from the literature we believe that there is a general explanation for the genesis of these tumors, namely, that they arise from remnants of the embryonal urogenital apparatus. This concept would account for the histological structure, as well as diversity of histological elements of these tumors. It would also account for the fact that, with few exceptions, well established tumors found in the adult urogenital organs have also been found free retroperitoneally. In fact this concept would seem to unify all

\* Received for publication May 11, 1931.

degeneration the glomeruli and secretory tubules degenerate, but the excretory tubules and corresponding part of the collecting duct become further modified to establish connection with the genital gland, forming the epigenitalis and the paragenitalis. For this function there must be a urogenital union which occurs between the rete of the genital gland and the excretory (transverse) tubules of the mesonephros.

4. *Metanephros*: The metanephros develops from two systems: (1) the metanephrogenic tissue which is the lower 1 to 2 segments of the nephrogenic cord, and (2) the ureter bud which appears from the Wolffian duct just proximal to the cloaca. The glomeruli and secretory tubules are from the former, and the collecting tubules, pelves and ureters are from the latter. The ureter anlage first appears in the region of the fifth lumbar segment in an embryo of 5.3 mm. greatest length. This ureter bud is capped by the metanephrogenic tissue which becomes displaced from the nephrogenic cord by dorsal growth of the ureter. After reaching a dorsal position growth is in the cephalic direction, reaching the first to second lumbar segment. The metanephros is then in the approximate location of the adult kidney. Complete development of the metanephros does not occur until after birth. There is no degeneration of this structure.

5. *Genital Cell*: The origin of the genital cell is disputed, but in general two theories of significance are offered. The first theory is formation from the celomic epithelium "in loco." Authors proposing this theory think that the celomic epithelium possesses the potency of forming reproductive cells. A second theory is the derivation of the genital cell directly from segmentation cells. Hence, it becomes necessary for the genital cells to migrate from their site of formation into that part of the celomic epithelium which is destined to become the germinal epithelium. Here, it seems, genital cells might easily stray to foreign locations.

6. *Genital Gland*: The genital gland forms from the celomic epithelium covering the ventral part of the urogenital fold. The gland is at first indifferent. The anlage is first seen in a 4.9 mm. embryo, located between the first and third dorsal segments. It appears as a thickening of the germinal epithelium which becomes multilayered. Later, the epithelium invades the mesenchyme which compresses it and pushes the mesonephros dorsally. The genital fold is then formed by evaginations of the celom, and the genital gland and

develops in embryos between the 14 and 23 segment stages and reaches the cloaca in an embryo with 27 pairs of segments. This duct is located in the parietal mesenchyme just beneath the ectoderm. The pronephric segments completely degenerate, but the excretory duct participates in the formation of the mesonephros.

2. *Urogenital Fold*: This fold contains the mesonephros, genital gland, Wolffian duct and Müllerian duct. A fold forms because there is not sufficient room retroperitoneally for the developing structures and they invaginate into the celomic cavity. The fold is first seen in a 2.5 mm. embryo, when it extends from the fifth cervical to the eighth dorsal segment. Growth and degeneration are caudad and by the 50 mm. stage the fold extends only from the fourth to fifth lumbar segments. These urogenital folds are found on either side of the midline and undergo certain changes.

(a) The fold becomes divided into four parts by evaginations of the celomic cavity (a lateral tubular part, a ventral genital part, a dorsal glandular part and a medial mesentery portion). The Müllerian duct, genital gland and mesonephros develop in the first three parts respectively.

(b) Caudad, the two folds unite to form the urogenital cord, which divides the pelvis into an anterior and posterior part. The union of the Müllerian ducts is thus permitted. The relationship of the various structures in the fold can best be seen in Diagrammatic Drawing 1.

3. *Mesonephros*: The mesonephros forms from the middle and major portion of the nephrogenic cord, overlapping cranially to some extent with the pronephros and extending caudad to the twenty-sixth or twenty-seventh primitive segment. It extends over approximately 18 segments and in all about 83 tubules form. This first appears in the 2.5 mm. stage. Growth and degeneration can be followed in Text-Fig. 1. The first anlage is a vesicle which forms the principal tubule. It establishes connection with the Wolffian duct laterally. A supplemental tubule forms mesially later, making an S curve and uniting with a glomerulus. Degeneration is divided into two stages, the first of which is complete in the 21 mm. embryo. It includes the upper 57 tubules, no part of which participate in the formation of adult structures. Thus, at this stage (21 mm.), the mesonephros consists of the lower 26 tubules and extends from the second to the fourth lumbar segments. In the second period of

mesogenital become definite structures. The indifferent gland is a homogeneous mass of epithelial cells in intimate contact with, but definitely separated from, the collecting tubules of the mesonephros. The extent of growth and degeneration can best be followed in Text-Fig. 1. The maximum extent of this structure is 14 segments, but the upper 10 to 12 degenerate and eventually only 2 to 4 remain to form the adult organ. Differentiation into the male sex gland begins at about the 13 mm. stage, or into the female gland at a somewhat later stage. It seems unnecessary to say more than that the differentiated sex organ is formed from this epithelial mass and that urogenital union occurs as mentioned above.

7. *Müllerian Duct*: The ostium of the Müllerian duct is formed by an invagination of the celomic epithelium into the summit of the urogenital fold. The tubal portion forms from a caudal growth of this blind end. The anlage of the ostium is first seen in an embryo of 10 mm. greatest length at the level of the third dorsal segment. The blind end grows caudad in the lateral (tubal) portion of the urogenital fold to reach Müller's tubercle, located in the dorsal wall of the vesico-urethral anlage. This occurs in embryos between 21 and 27 mm. greatest length. The two ducts unite in the urogenital cord in embryos of about 28 mm. length. In the female there is no degeneration, but descent occurs as the body of the embryo grows cranially, and later rotation occurs. The entire structure remains to form the fimbria, fallopian tube, uterus and vagina. In the male, degeneration is complete except for the hydatid of Morgagni and the sinus pocularis, male vagina.

8. *Adrenal Cortex*: The adrenal cortex develops from the celomic epithelium, the first traces appearing in the 6 mm. embryo. The epithelium thickens and invades the mesoderm ventrolateral to the aorta, and mesial to the urogenital fold. With increase in size of the gland it projects into the celomic cavity, forming the suprarenal ridge. This is between the mesonephros and the mesentery. In an 8 mm. embryo the glands are already definite organs completely separated from the celomic epithelium, and vascularization occurs in the 9 mm. stage. Differentiation of the cortex into three layers takes place rather late. No degeneration occurs. Accessory nodules of cortical tissue are of frequent occurrence and may be found in the following locations: (a) suprarenal capsule; (b) broad ligament; (c) hilum of sex glands; (d) region of the spermatic vessels; (e) kid-

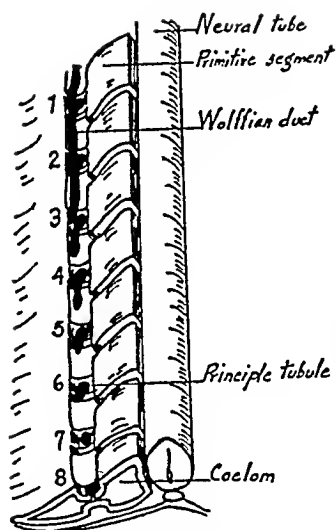
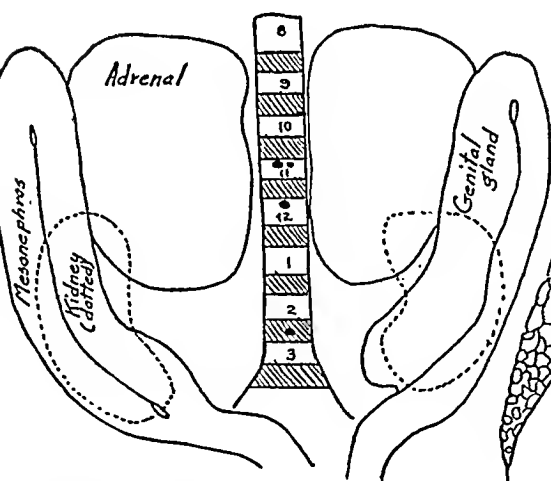
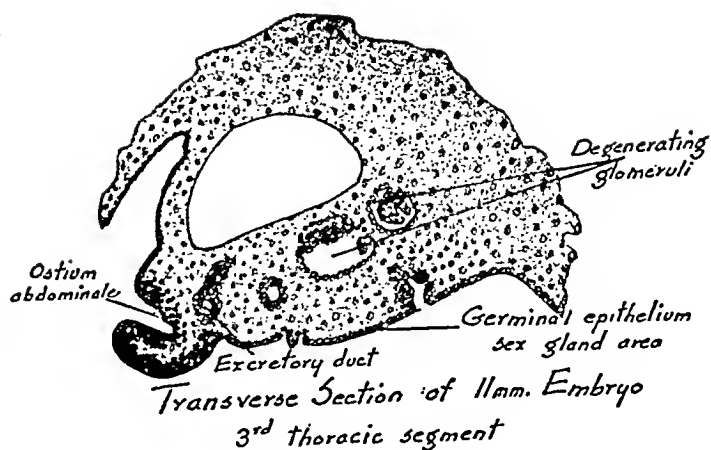
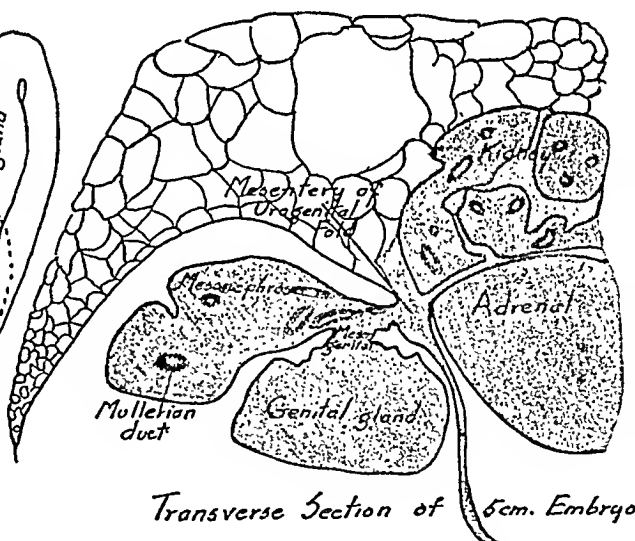


Diagram of Development  
of Pronephros



Reconstruction from 19.4mm. Embryo



Transverse Section of 6cm. Embryo

# DIAGRAMMATIC DRAWING I

Relationships of urogenital organs. Compilation from Keibel and Mall



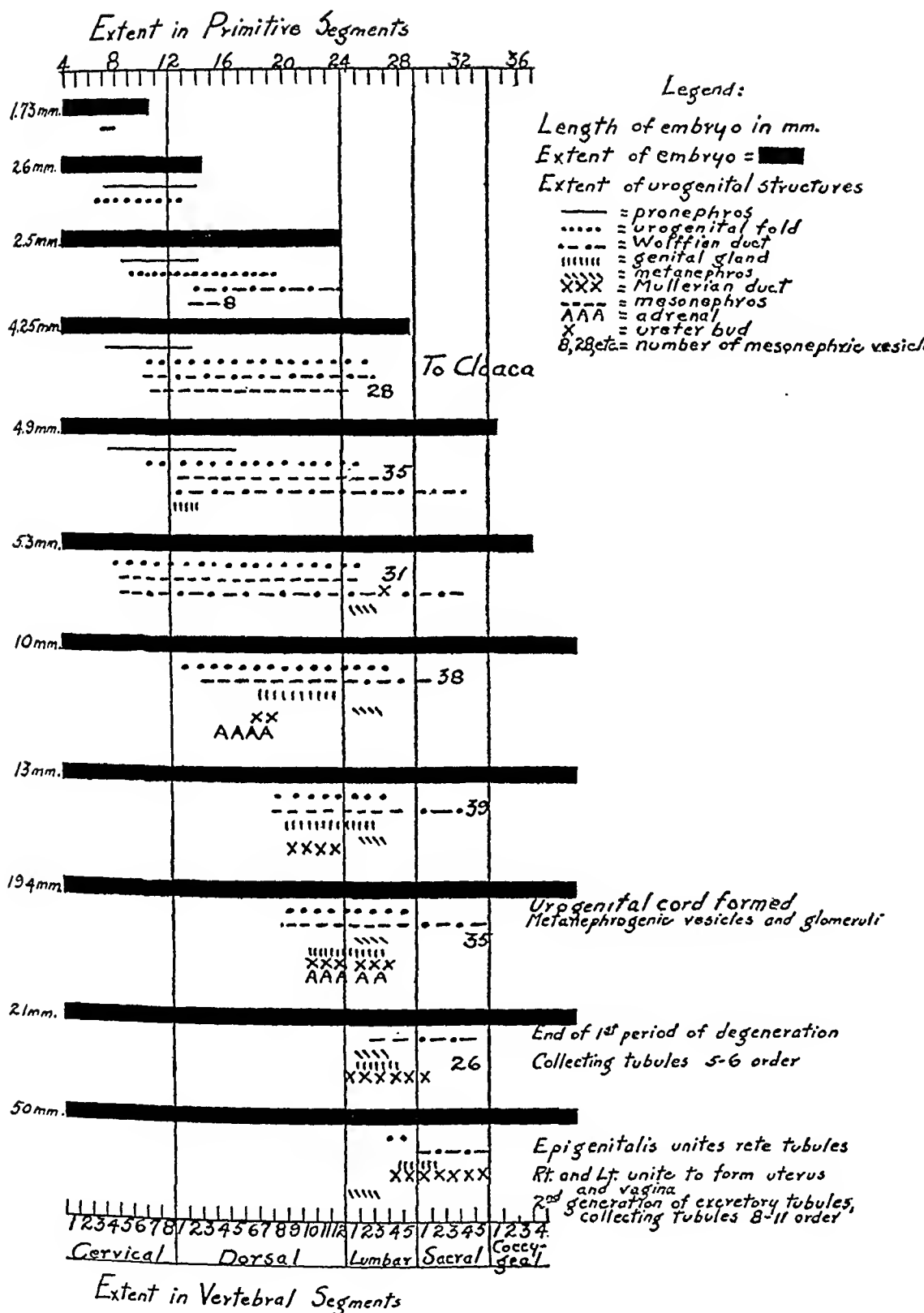
ney; (f) liver, etc. Theories as to the origin of these accessory nodules are: (a) breaking and displacement of cortex by invasion of medulla; (b) inclusion of cortex in adjacent developing organs; (c) formation of cortical tissue from transverse tubules of the mesonephros, and (d) inclusion of celomic epithelium which has the potency to form adrenal cortex.

### PRESENTATION OF MATERIAL

Information concerning tumors of the urogenital apparatus is widely scattered in the medical literature of the world. It is to be found in articles usually comprising single case reports. Some authors have chosen to interpret the pathogenesis of their peculiar case, while others have contented themselves with the report of an unusual case. More attention has been given the subject in Germany than in all other countries combined. Only four references to this group of tumors were found in the American medical literature. In three of these four instances, according to the authors, the tumors arose from the mesonephros, while no definite interpretation was given for the small round cell tumor in the fourth report further than to assume that it was a rapidly growing portion of a mixed tumor because it arose in the kidney region. Nowhere has there been an attempt to survey the morbid anatomical conditions on the broad basis of anomalous unabsorbed residues in the development of the urogenital apparatus.

A summary of the information which has accrued can be concretely presented in tabular form. A natural division of the material is immediately apparent. Similar to tumors in the adult urogenital organs, the tumors from their remnants are both solid and cystic. The cystic and solid tumors are segregated in two tables for consideration.

The tabulation shows that cystic tumors of this group have been known since 1871, when a retroperitoneal dermoid teratoma similar to the dermoid teratoma of the ovary was described. It is natural that this type of tumor should be recognized, first because hair and well formed bone are easily identified grossly and the grumous greasy material of epithelial cysts is very characteristic. In 1889 Bassini first described a tumor which, if located in the ovary, would have been diagnosed cystadenoma of the ovary. Frank, in 1894, after giving a description of a similar tumor, stated that the lesion



TEXT-FIG. 1

Status of urogenital apparatus in embryos from 1.73 to 50 mm. in length.

TABLE I  
*Review of Unattached Cystic Tumors of the Urogenital Apparatus Reported in the Literature*

Author	Age (years)	Sex	Duration	Location	Histology	Histogenesis	History	Estimated weight (gm.)
Howship (1871). <i>Deutsche Ztschr. f. Chir.</i> , (Cit.) 1901, 61, 1	2	F	?	LL	Dermoid teratoma	?	Died	Large
Hosmer (1880). <i>Deutsche Ztschr. f. Chir.</i> , (Cit.) 1901, 61, 1	8 mo.	F	?	RL	Dermoid teratoma	?	?	1000
Zweifel. <i>Zentralbl. f. Gynäk.</i> , 1888, 12, 439	18	F	?	LL	Hairy skin	?	Removed, well	?
Bassini. <i>Zentralbl. f. Gynäk.</i> , 1889, 13, 640	37	F	3 yrs.	LL	Ovarian cystoma	?	Removed, well	1100
Frank, E. <i>Wien. klin. Wchnschr.</i> , 1894, 7, 649	37	F	6 yrs.	RL	Ovarian cystoma	Accessory ovary	Removed, well	10,000
Gude, H. (1898). <i>Deutsche Ztschr. f. Chir.</i> , (Cit.) 1901, 61, 1	9	M	?	LL	Dermoid teratoma, infected	?	Died	?
Dowd, C. N. <i>Ann. Surg.</i> , 1900, 32, 515	41	F	8 yrs.	LUQ	Ovarian cystoma	Ovarian sequestrum	Removed, well	2000
Göbel, R. <i>Deutsche Ztschr. f. Chir.</i> , (Cit.) 1901, 61, 1	54	F	27 yrs.	LL	Dermoid teratoma	Bigeminal inclusion	Metastases after 26 yrs.	1000
Seitz, L. <i>Deutsche Ztschr. f. Chir.</i> , (Cit.) 1901, 61, 1	29	F	3 wks.	RL	Ovarian cystoma	Accessory ovary	Removed, well	4500
König. <i>Deutsche Ztschr. f. Chir.</i> , (Cit.) 1901, 61, 1	40	F	?	RL	Hair	?	Removed, well	2000

was identical with cystadenoma of the ovary. The theory was then launched that these unattached cystadenomas had their origin in accessory ovaries. Goebel, in 1901, in the interpretation of the histogenesis of his case of dermoid teratoma came to the conclusion, after a lengthy discussion of teratology in which the possibility of a sex cell origin was included, that the tumor under consideration had its origin in a bigerminal inclusion. Up to the present time this interpretation has not been further clarified. On the other hand, the cystadenoma type of lesion has apparently been put upon a firm foundation. Subsequent to Frank's noting the similarity of retroperitoneal cystadenomas and cystadenomas of the ovary, glomeruli and renal tubules have, at times, been found in the walls of these cysts. It seems logical to conclude, therefore, that these cysts were mesonephric rather than ovarian in origin. This is the reaction of those who have studied this subject most thoroughly, and the idea of accessory ovary origin has disappeared from the recent medical literature.

Information about solid unattached tumors of this embryological structure began to accumulate at a somewhat later date than that of the cystic tumors and is, even now, very incomplete and unsatisfactory. In 1901 Goebel described a tumor in the left upper quadrant, which upon section presented typical adrenal histology. The adrenal was present on one side of the tumor and was uninvolved. In the same year Chiari and Weise each described an alveolar carcinoma which had arisen independently of adult adrenals. They interpreted the origin from adrenal rests because the cells resembled those of the adrenal. Today, similar tumors found in the kidney would be diagnosed renal cell carcinomas. Verocay in 1906 found a mixed tumor retroperitoneally in the left upper quadrant, which he interpreted as a metastasis from a dermoid teratoma of the ovary. It would appear that the two tumors were independent, because behind the lesser peritoneal cavity would be an unusual place for a metastasis from the ovary and because dermoid teratomas of ovary rarely metastasize. A tumor similar to the one in question, if found in the kidney, would be diagnosed an embryonal tumor from the renal blastema. Chorio-epithelioma-like tumors have been reported several times without relationship to pregnancy, sex or fecundation. They have been found in the bladder, expelled from the vagina of a 3 year old child, in the retroperitoneal region, liver,

Author	Age	Sex	Duration	Location	Histology	Histogenesis	History	Estimated weight (gm.)
Maury, J. M. <i>Surg. Gynec. Obst.</i> , 1918, 26, 663	28	F	14 mo.	LL	Tubules, glomeruli	Mesonephros	Well	2000
Staehlin, E. <i>Ann. Surg.</i> , 1915, 61, 312	61	M	7 yrs.	RL	Adenocystoma	Mesonephros	Rupture, implants, death	4000
Hinman, F., Gibson, T. E., and Kutzman, A.A. <i>Ann. Surg.</i> , 1924, 79, 762	27 mo.	M	6 mo.	LL	Tubules, glomeruli	Mesonephros	Well	600
Pettis, J. H. <i>Physician &amp; Surg.</i> , 1913, 35, 27	39	F	10 mo.	LUQ	Pseudomucinous cystadenoma	Mesonephros	Died, metastases	700
Moore, F. C. <i>M. Chron.</i> , 1902, 4, 337	38	M	21 mo.	LUQ	Pseudomucinous cystadenoma	Mesonephros	Died	4000
Moore, F. C. <i>M. Chron.</i> , 1902, 4, 337	64	M	7 yrs.	LUQ	Pseudomucinous cystadenoma	Mesonephros	Died, metastases	4000
Monprofit. <i>Gaz. méd. de Paris</i> , 1904, 4, 121	?	F	8 yrs.	LUQ	Pseudomucinous cystadenoma	Mesonephros or ovary	Well	5000
Jacquot, C., and Fainse, C. <i>Rev. de gynéc., Paris</i> , 1913, 20, 551	18	F	Indefinite	LUQ	Serous cystadenoma	Mesonephros	Died, peritonitis	3500
Hedinger. <i>Virchows Arch. f. path. Anat.</i> , 1902, 167, 29.	40	F	Found at post-mortem	LL	Serous cystadenoma	Ovary	None	500
Lockwood (1898). <i>Rev. de gynéc., Paris</i> , (Cit.) 1913, 20, 551	21	F	2 yrs.	LUQ	Serous cystadenoma	Mesonephros	Removed, well	?
Lockwood (1898). <i>Rev. de gynéc., Paris</i> , (Cit.) 1913, 20, 551					Serous cystadenoma	Mesonephros	Removed, well	?

Helbing, C. <i>Deutsche Med. Wchnschr.</i> , 1901, 27, 228	51	M	4 mo.	LL	Papillary adenoma	?	Died	1500
Verocay, J. <i>Ztschr. f. Heilk.</i> , 1906, 27, 176	32	M	4 yrs.	LUQ	Adenocystoma	?	Inoperable	?
Niosi, F. <i>Virchows Arch. f. path. Anat.</i> , 1907, 190, 217	48	F	1 mo.	LLQ	Renal tubules, glands, chorio- epithelium, adrenal-like cells	Wolffian body	Removed, well	1000
Pullmann, W. <i>Arch. f. klin. Chir.</i> , 1909, 89, 357	41	F	1 mo.	LLQ	Ovarian cystoma	Ovarian rest	Removed, well	2000
Oser, E. G. <i>Arch. f. klin. Chir.</i> , 1911, 95, 131	38	F	9 yrs.	LUQ	Ovarian cystoma	Anlage of ovary	Infection and death	1500
Naegeli, T. <i>Beitr. z. klin. Chir.</i> , 1918, 110, 425	22	F	4 yrs.	RLQ	Ovarian cystoma	Supernumerary ovary	Removed, well	1000
Dubs, J. <i>Arch. f. klin. Chir.</i> , 1919, 111, 860	36	F	2 yrs.	RA	Necrotic wall	?	Removed, well	6000
Elder, J. M. <i>Canad. M. A. J.</i> , 1920, 10, 273	2	F	3 wks.	RA	Connective tissue	Wolffian body	Marsupial- ized, well	?
Schweitzer, G. B. <i>Monatschr. f. Geburtsh. u. Gynäk.</i> , 1920, 52, 171	41	F	6 yrs.	LUQ	Ovarian cystoma	Wolffian anlage	Removed, well	1500
Schweitzer, G. B. <i>Monatschr. f. Geburtsh. u. Gynäk.</i> , 1920, 52, 171	40	F	4 mo.	LUQ	Ovarian cystoma	Wolffian anlage	Removed, well	1700

LUQ = left upper quadrant.  
RUQ = right upper quadrant.

LL = left lateral abdomen.

RL = right lateral abdomen.

RA = right abdomen.

LLQ = left lower quadrant.

RLQ = right lower quadrant.

TABLE II  
Review of Unattached Solid Tumors of the Urogenital Apparatus Reported in the Literature

Author	Age (years)	Sex	Duration	Location	Histology	Histogenesis	History	Estimated weight (gm.)
Göbel. <i>Deutsche Ztschr. f. Chir., (Cit.)</i> 1901, 61, 1	25	M	5 mo.	LUQ	Adrenal cell carcinoma	Adrenal present, adrenal rest	Operation, death	1500
Chiari, H. <i>Deutsche Ztschr. f. Chir., (Cit.)</i> 1901, 61, 1	44	M	6 mo.	RUQ	Alveolar carcinoma	Adrenal rest	Recurrent, operation, death	?
Verocay, J. <i>Ztschr. f. Heilk., 1906, 27, 176</i>	48	F	2 mo.	RA	Glands and sarcoma	Metastasis from dermoid	Intra-abdominal, hemorrhage, death	2000
Verocay, J. <i>Ztschr. f. Heilk., 1906, 27, 176</i>	3	F	?	MA	Alveolar carcinoma	Misplaced kidney tissue	Removed, well	1300
Weiss, B. <i>Deutsche Ztschr. f. Chir., (Cit.)</i> 1901, 61, 1	27	F	4½ yrs.	LUQ	Alveolar carcinoma	Adrenal present, adrenal rest	Operation, death	2000
Göbel. <i>Deutsche Ztschr. f. Chir., (Cit.)</i> 1901, 61, 1	67	M	3 mo.	LL	Alveolar carcinoma	Adrenal present, adrenal rest	Operation, death	1500
Lubarsch. <i>Arb. a. d. pathl. Anat. abt. d. Kgl. Hyg. Anat. z. Posch., 1901, p. 230</i>	12	F	?	Uterus	Chorio-epithelioma		Virgin	Large
Bostroem. <i>Verhandl. d. deutsch. pathl. Gesellsch., 1902, 5, 212</i>	30	M	2 mo.	ML	Chorio-epithelioma		Died, testes normal	Large

	55	F	3 yrs.	RL	Serous cystadenoma	Accessory ovary	Died	?
Kast, L., (1904). <i>Rev. de gynéc., Paris</i> , ( <i>Cit.</i> ) 1913, 20, 551								
Albarrañ (1903). <i>Rev. de gynéc., Paris</i> , ( <i>Cit.</i> ) 1913, 20, 551	10 mo.	F	3 mo.	RL	Cystadenoma, Wolfian tubules	Mesonephros	Well	1000
Heyrowski (1908). <i>Rev. de gynéc., Paris</i> , ( <i>Cit.</i> ) 1913, 20, 551	26	M	20 yrs.	RUQ	Pseudomucinous cystadenoma	Mesonephros	Marsupial- ized, re- moved 5 yrs. later, re- curred 5 yrs. later, died	Massive
Guillemin, A. <i>Bull. Soc. d'obst. et de gynéc. de Paris</i> , 1922, 20, 747	47	F	1 mo.	Pelvis	Cystadenoma	Mesonephros	Removed	1500
Madier, J., and Nathan, M. <i>Bull. et mém. Soc. nat. de chir.</i> , 1924, 50, 200	2½	?	3 wks.	LL	Cystadenoma	Mesonephros	Removed	700
Sacerdote, G. <i>Arch. ital. di chir.</i> , 1926, 16, 122	57	F	2 yrs.	Inguinal canal	Cysts, glomeruli, tubules	Mesonephros	Removed	100
Lawen, A., and Biebl, M. <i>Beitr. z. klin. Chir.</i> , 1928, 144, 505	18	F	1 yr.	RL	Granulation tissue, glomeruli	Mesonephros	Removed, well	700
Romeo, M. <i>Ann. ital. di chir.</i> , 1928, 7, 313	35	F	3 yrs.	LL	Three cavities lined by con- nective tissue	Mesonephros	Removed	8000
Brouha, M., and Gosselin, O. <i>Bruxelles- méd.</i> , 1929, 9, 831	44	F	5 yrs.	RL	Cystadenoma	Mesonephros	Removed	5000



kidney, mediastinum, and pineal gland. Smith recently described a case of a round cell tumor in the kidney region. He did not liken it to the so-called embryoma of the ovary and of the testicle, but he did suggest a totipotent cell as the etiology. The basis for this interpretation was that tumors of the kidney are probably all mixed tumors. Clarke<sup>1</sup> has recently reviewed the theories relating to the histogenesis of tumors containing endometrial-like tissue. He, as well as other authors cited by him, are satisfied that all tumors made up of endometrial-like tissue do not have their origin in implantation of bits of endometrium as described by Sampson. Among several other modes of origin, embryonic Müllerian tissue was suggested. The literature also contains a number of accounts of smooth muscle neoplasms arising retroperitoneally without organ attachment. Could they have arisen from remnants of the Müllerian apparatus? Mixed tumors of mesenchymal origin have also been observed in this region and they occur more frequently than where embryonal structures do not exist. They, too, may have their origin in the urogenital apparatus.

Table III presents the seventeen cases which we have had the opportunity of studying. Fifteen of the cases entered the University Hospital during the last seven years, while two were recognized among the tissues sent to the University by physicians of the state. Sixteen of the tumors were quite independent of the adult genito-urinary organs. In two instances there was invasion of the kidney, which was obviously secondary to a much larger and older tumor of the urogenital fold. While the origin was not so clear in a third case, we believe that an extrarenal origin was the more likely. Case 17 cannot be shown to be urogenital in origin, but it is similar to fibroblastic tumors of mesothelial-lined surfaces such as pleura, synovium and peritoneum, and we have reasoned that it may have had its origin from the mesothelium of the urogenital fold. It had completely surrounded the kidney.

The tabulation comprises a rather heterogeneous group of tumors. Three are cases with epithelial cysts. Two were single cysts and because of the nature of their contents were probably epidermoid. The third was a composite dermoid containing abundant hair and a large flat bone. There were two tumors of typical chorio-epitheliomatous histology. Four massive, friable, solid tumors made up of large round cells were comparable to the so-called embryomas of the

Djiewitzki, <i>Virchows Arch. f. path. Anat.</i> , 1904, 178, 451	75	F	?	?	Chorio- epithelioma	Virgin	?
Ritchie, <i>J. Obst. &amp; Gynec. Brit. Emp.</i> , 1904, 4, 65	24	M	10 days	Anterior medi- astinum	Chorio- epithelioma ? and teratoma	Suffocated	Large
Marx, H. <i>Beitr. z. path. Anat. u. z. allg. Pathol.</i> , 1904, 36, 385	52	M	?	Liver	Chorio- epithelioma	Death	?
Bonney, V. <i>Tr. Path. Soc. London</i> , 1907, 58, 9	69	M	3 mo.	Great omen- tum		Tapped for 4000 cc. bloody fluid, death	1600
Bock, <i>Tr. Path. Soc. London</i> , 1907, 58, 9	12	F	?	Expelled from vagina	Hydatid mole	Virgin, 4th menses	20
Askanazy, <i>Verhandl. d. Deutsch. path. Gesellsch. zu Stuttgart</i> . (Cf. Bonney, p. 30.)				Pineal gland	Chorio- epithelioma		
Smith, L. H. <i>Northwest Med.</i> , 1927, 26, 289	17 mo.	F	2½ mo.	LUQ	Solid round cell tumor	Death, metastases	?
Masao, M. <i>Mitt. u. allg. Pathol. u. path. Anal.</i> , 1928, 4, 144	39	F	3 mo.	Right kidney	Chorio- epithelioma	Hydatid mole 2 years previous, death, metastases	300
Masao, M. <i>Mitt. u. allg. Pathol. u. path. Anal.</i> , 1928, 4, 144	45	F	2 mo.	Right kidney	Chorio- epithelioma	Operation, death, no metastases	600

LUQ = left upper quadrant.  
RUQ = right upper quadrant.

LL = left lateral abdomen.  
RA = right abdomen.

MA = midabdomen.  
ML = midline.

testicle. The histology of Case 11 and Case 12 is identical with two types of carcinoma of the ovary pictured in Ewing's "Neoplastic Diseases." Case 10 was quite similar morphologically to carcinosarcoma of the adult endometrium. Three cases, because of rather mixed histology, including typical Grawitz tumor histology, glomeruli, bone and alveolar carcinomatous areas, we consider were mesonephric in origin. Case 16 reproduces quite accurately adult renal tubule histology. Case 17 was included because it is a fibrosarcoma which may have had its origin in the mesothelium of the urogenital fold.

### CASE REPORTS

**CASE 1. History:** J. E. (E 1902), a white male of 78 years had pain in the upper right quadrant in September, 1929, a pain which was aggravated by drawing up the legs. Another attack occurred on February 20, 1930, which lasted two days. A third attack occurred recently which lasted a week and was especially severe. He entered the hospital March 13, 1930 after a 15 pound loss of weight. He had had attacks of abdominal pain forty, and again fourteen, years ago.

The patient had some distress when in the dorsal position. There was a large, tender, globular mass which extended from three fingerbreadths below the costal margin up under the right costal margin.

The hemoglobin was 75 per cent, red blood cells 4,280,000, and there were 13,600 to 18,000 white blood cells.

Roentgenological examination showed an incisure of the greater curvature with a niche opposite it indicating gastric ulcer. A shadow in the right upper quadrant was interpreted to be a tumor. The chest was negative. Cystoscopic examination showed a bifid type of pelvis, but it was felt that the mass was independent of the kidney even though the upper calyces of the right kidney were somewhat distorted. At operation cholecystitis and cholelithiasis were demonstrated in addition to an independent large cystic mass. 1600 cc. of brownish material containing a great deal of brownish amorphous material and many cholesterol crystals were evacuated from the cyst. No hair was noted. A piece of cyst was removed for histological study.

**Histological Examination:** The wall of the cyst was made up of a thick zone of granulation tissue in which were many cholesterol clefts. There was also a heavy infiltration of polymorphonuclear leucocytes. Mononuclear cells and hemosiderin pigment were also present (Fig. 1).

**Subsequent Course:** The cavity for the most part disappeared and the patient left the hospital with a slight amount of drainage from the operative wound.

**CASE 2. History:** J. E. (B 2061), a white male of 51 years entered the hospital with a probable diagnosis of perinephritic abscess. The acute illness began

TABLE III

Review of Unattached Tumors of the Embryonal Urogenital Apparatus from 17 Cases in this Report

Case No.	Hosp. No.	Age (years)	Sex	Duration	Location	Histogenesis	Function	Original Diagnosis	History
1	E 1902. 1930	78	M	7 mo.	RUQ	?	o	Infected epithelial cyst	Operation, well
2	B 2061. 1927	41	M	36 yrs.	LUQ	Sex cell	o	Infected dermoid cyst	Death
3	E 8941. 1930	67	M	26 yrs.	LUQ	?	o	Infected epithelial cyst	Death
4	Outside. 1930	26	M	3 mo.	LUQ	Sex cell	o	Chorio-epithelioma	Death
5	B 11067. 1927	26	M	1 mo.	LL	Sex cell	o	Chorio-epithelioma	Death
6	D 7405. 1929	24	M	1½ yrs.	ML	Sex cell	o	Indifferent carcinoma	Death
7	C 5985. 1928	2	F	3 mo.	LUQ	Sex cell	o	Neuroblastoma	Death
8	C 3247. 1929	37	M	1 yr.	ML	Sex cell	o	Indifferent carcinoma	Death
9	59861. 1924	3	F	3 yrs.	LL	Sex cell	o	Indifferent fibrosarcoma	Death
10	Outside. 1930	2	F	?	LUQ	Müllerian apparatus?	o	Tumor of urogenital apparatus	Inoperable, death
11	C 1737. 1928	70	F	6 mo.	LL	Rete ovarii	o	Adenocarcinoma	Death
12	E 2426. 1930	37	F	1½ yrs.	LUQ	Rete ovarii	o	Adenocarcinoma	Metastases
13	D 232. 1929	48	M	1 mo.	LUQ	Mesonephritic tubules	o	Adrenal cell carcinoma	Removed Jan. 1929, well Dec. 1930
14	E 1661. 1930	51	F	2 yrs.	RUQ	Mesonephritic tubules	o	Urogenital apparatus tumor	Metastases
15	D 6964. 1929	33	F	1 yr.	LUQ	Mesonephritic tubules	o	Adrenal cell carcinoma	Death
16	E 6888. 1930	66	M	3 mo.	RL	Metanephritic tubules	o	Renal cell carcinoma	Death
17	E 2084. 1930	41	M	10 mo.	LUQ	?	o	Fibrosarcoma	Death

LUQ = left upper quadrant.  
RUQ = right upper quadrant.

LL = left lateral abdomen.  
RL = right lateral abdomen.

ML = midline.

CASE 3. *History:* W. S. (E 8941), a white male 67 years old, had had an attack of pain in the upper abdomen in the summer of 1884. The onset was sudden and the character of the pain was designated as knife-like. It came on while working and lasted twenty-six hours. October of the same year there was a similar attack, while a third attack was suffered in December. He has since had "bloating spells" and discomfort in the upper left abdomen. In 1904 he had a severe trauma to the chest. Several months following this, 3000 cc. of pus were removed from the left upper abdomen through a posterior approach. He was very weak and ill at this time and his weight dropped to 98 pounds. Since then, there have been neuralgic pains in the left side. Since 1928, the pain and distress in the upper abdomen have become more severe. In July, 1930, the patient began losing appetite. This was later followed by nausea and then by vomiting. His weight dropped from 140 to 107 pounds.

Except for senility the physical findings were limited to the abdomen. A hard mass extended two fingerbreadths below the left costal margin which was 13 cm. in transverse diameter and extended from the midaxillary line to the lateral border of the sternum. The mass was hard and it descended with respiration.

The urine showed a trace of albumin and a few granular casts. Hemoglobin 84 per cent, red blood cells 4,000,000 and white blood cells 9,800. The blood Wassermann was negative. No free acid appeared in the gastric content even after the administration of histamine.

Roentgenological examination of the colon was negative. The stomach contour was normal and the duodenal cap well outlined. The esophagus arched over the mass which, in turn, retarded the flow of barium into the stomach. Pyclograms showed normal pelves. A large calcified cystic mass was stereoscoped anteriorly to the kidney. Whether or not this mass arose in the kidney could not be determined (Fig. 2). At operation the mass was firmly adherent in the left upper quadrant to the stomach, kidney, spleen and pancreas. An attempt was made to remove it, but the patient died during the operation.

*Postmortem Examination:* A 1200 gm. mass was found situated retroperitoneally behind the lesser peritoneal sac. The wall was made up of old granulation tissue and was markedly calcified, measuring 0.5 cm. in thickness. There was but one cavity which was filled with brownish, thin material. It appeared to be an old epithelial cyst, the epithelium of which had been destroyed by infection. There was no hair. There were firm adhesions to the stomach, spleen, left kidney and pancreas. These organs were, however, absolutely distinct from the mass. The splenic artery coursed along one surface of this mass.

CASE 4. *History:* A male, 26 years of age, came under observation with the complaint of exhaustion. He had lost 15 pounds in weight. There was indefinite distress in the upper abdomen, which was worse after eating. A smooth tumor was revealed in the epigastrium. Both testicles were in the scrotum and appeared to be normal.

At operation the mass was back of the lesser peritoneal cavity and was ap-

February 22, 1927, with sharp pain in the lower abdomen. No definite localization of the pain could be pointed out by the patient. There was much bloating and the abdomen was tender. He was taken to the hospital and the local physician thought that the patient might have gall-bladder disease or appendicitis. Temperature varied from 100° to 103° F. He entered the University Hospital February 27, 1927. At that time the pain had localized well over the left kidney region. The patient stated that when he was 4 years old he had a tumor mass in the left side of the abdomen which was quite large. A physician thought that it was attached to the spleen. It disappeared in six months without treatment. He ran a fever and was undernourished until the age of 9, when he began to develop normally. At 21 years he had several hemorrhages, apparently from the gastro-intestinal tract.

The positive points in the physical examination were slight fullness in the left flank and tenderness in the left flank and posteriorly.

The hemoglobin was 87 per cent and the whiteblood cells numbered 17400. The urine had a normal gravity and was free from pathological constituents.

Roentgenological examination showed the left diaphragm to be elevated two intercostal spaces. Air and fluid were demonstrated in an abnormal cavity below the diaphragm. February 28, 1927, the cavity was opened, liberating a cupful of pus which contained considerable detritus. The wall appeared to be discolored granulation tissue. The temperature remained elevated and tenderness beneath the costal margin increased. The patient was reoperated upon March 9, 1927. The stomach and gastrocolic omentum were adherent to the mass. Another cavity of foul pus was opened up. At neither operation was the relationship of the lesion to the viscera, nor the nature of the lesion, ascertained. After the second operation, the tenderness of the abdomen became diffuse and the leucocytes rose to 31,000. March 16, 1927, the anterior wound discharged an amorphous piece of tissue containing many long hairs. Drainage through the anterior wound was profuse and it irritated the skin. A test of the drainage for bile proved positive. The patient died on March 17, 1927.

*Postmortem Examination:* The body was slightly rigid, icteroid and poorly nourished. The skin about the two surgical incisions, each of which contained drainage tubes, was macerated. The small bowel was agglutinated by fibrin. The peritoneum was lusterless and injected. A large indistinct mass occupied the region of the lesser peritoneal sac. It was multilocular and the walls were made up of granulation tissue. There were firm adhesions to the spleen, stomach and left kidney. 10 cm. above the pylorus where the mass was firmly adherent to the stomach was an ulcer 3 cm. in diameter which had perforated into the mass. All cavities contained hair and one cavity contained a flat bone which measured 3 by 1.5 cm. All organs showed evidences of intravascular infection.

*Histological Examination:* Sections of the lesion showed a granulation tissue wall which contained many cholesterol clefts and was heavily infiltrated with inflammatory cells, chiefly polymorphonuclear.

CASE 6. *History:* C. S. (D 7405), entered the hospital October 11, 1929. He had been well until three months previously, when he had vomiting spells every few days. The vomiting relieved an overcrowded feeling of the abdomen and was not associated with much discomfort or nausea. October 1 he visited a physician, who told him he had a tumor. Recently, he has eaten very little, lost 7 pounds weight and a great deal of strength.

There was a mass in the abdomen which measured 18 by 20 cm. It was firm, fixed and did not move with respiration. Veins were very prominent over the right abdomen but less prominent over the left. Testicles and lymph nodes were negative.

The hemoglobin was 70 per cent, red blood cells 4,000,000 and white blood cells 5,200. Wassermann negative. Blood urea nitrogen 42 mg.

On roentgenological examination the gastro-intestinal tract was negative except for being distorted by a large abdominal tumor. X-ray treatment caused a practical disappearance of the mass in two weeks' time, as far as physical examination was concerned.

He was discharged October 23, 1929, and returned on November 30, 1929. He had gained 26 pounds in weight. The abdominal tumor was almost the same as upon first admission. Weakness of head, neck, leg and arm muscles, which varied markedly from moment to moment developed, and death occurred December 7, 1929.

*Postmortem Examination:* A massive retroperitoneal tumor extended from the true pelvis to the third lumbar vertebra, which pushed all retroperitoneal structures forward. It was yellowish gray, firm and friable. The mesentery of the small gut revealed a few nodules 3 cm. in diameter. Both ureters were dilated and embedded in the mass. The right ureter showed a demonstrable constriction. The brain was negative grossly and histologically.

*Histological Examination:* The tumor mass resembled the solid, large, round cell tumor of the testicle.

CASE 7. *History:* M. R. (C 5985), a 2 year old, white female child was brought into the hospital September 22, 1928 because of fever, debility, weakness and pyuria. In July, 1928 the patient became irritable. August, 1928 she was feverish, temperature 102.4° F. Pus and albumin were reported in the urine on August 15, 1928. Since that time irritability increased. There was a desire to sit up and lean forward. She recently lost her vocabulary, and much strength. During the last week she was semicomatose. There was a mass in the left upper abdomen.

White blood cells ranged from 25,000 to 30,000. Nothing abnormal was found in the urine. X-ray after the colon was emptied revealed a large left kidney. Cystoscopy and ureteral catheterization showed a slight cystitis and no function of the left kidney.

The patient was operated upon the day of entrance in the hospital. An incision over the left kidney was made for the drainage of an infected hydronephrosis. As the kidney came into view a tumor involving its middle third was found. The kidney was removed. On histological examination large, usu-

proached through a hole made in the gastro-colic omentum. A tumor which was resilient and dark red was found retroperitoneally. As it could not be removed, a section was taken. Profuse bleeding followed the biopsy and the patient died ten days later.

*Histological Examination:* The tumor was in every way typical of a chorio-epithelioma.

CASE 5. *History:* H. I. (B 11067), a white male 26 years old, entered the hospital with shortness of breath, hemoptysis (abundant bright red blood), paralysis from the hips down, incontinence of urine and obstipation. The first trouble noted was weakness, in November, 1927. Tubercle bacilli were looked for in the sputum and the report was positive. On December 10, 1927, the patient developed severe backache which kept him awake nights almost constantly. December 18, he developed severe hemoptysis and at this time he was unable to move his left leg. On December 27 his right leg was practically paralyzed. He had incontinence and obstipation since December 22, 1927. His legs recently started to swell.

A dyspneic, emaciated, bedridden and cyanosed patient with hurried speech was observed on physical examination. The temperature was 97° F, pulse 120, and respiration 44. Teeth, throat and tongue were crusted with blood. Blood pressure 100/80. There were sonorous and sibilant râles throughout both chests. Edema extended from the toes to the midabdomen. Each calf measured 38.5 cm. in circumference. All sensations were decreased, ranging from 60 per cent to 0. Abdominal, knee jerk, tendon Achilles and plantar reflexes were absent. A slight flexion of the right thigh was all that the lower extremities could accomplish. No abdominal mass was palpated. The left testicle was not in the scrotum and had not been removed.

The hemoglobin was 55 per cent, and the red blood cells 3,270,000. Polymorphonuclear leucocytes 87 per cent. Blood culture, urine, and blood Wassermann negative.

On roentgenological examination there were large, tumorous opacities of both lung fields (Fig. 3). The skeleton was negative and indirect evidence of intracranial pressure was found. The patient died on Dec. 27, 1927.

*Postmortem Examination:* The lower extremities and the trunk were edematous and the body was emaciated. The left scrotum did not contain a testicle. Adhesions were present between the left lung and diaphragm. The combined weight of the two lungs was 4000 gm. The lung tissue was practically replaced by large, hemorrhagic masses (Figs. 4 and 5). The terminal ileum contained a few reddish nodules. A hemorrhagic mass which measured 10 x 7 cm. in diameter pushed up the right kidney, encircled the aorta, right ureter and spinal cord. The liver weighed 2600 gm. and contained numerous hemorrhagic nodules. The inferior vena cava was filled with tumor.

*Histological Examination:* Typical for chorio-epithelioma (Fig. 6).



pyelogram was negative. The left catheter placed in the left kidney pelvis drained 27,000 cc. of urine in forty-eight hours. The blood chemistry determinations were as follows:

Date	Urea nitrogen	Uric acid	Creatinine
	mg.	mg.	mg.
5/19/28.....	70.3	8.7	17.5
5/21/28.....	58.0	7.5	12.2
5/22/28.....	57.4	6.3	10.4
5/24/28.....	38.3	5.3	5.9
5/26/28.....	42.7	5.1	3.6
5/28/28.....	26.3	5.9	2.7
5/31/28.....	42.0	5.4	3.5
6/ 2/28.....	53.9	5.8	3.2
6/ 4/28.....	47.6	5.5	2.9
6/ 8/28.....	18.2	5.7	1.1

X-ray therapy was given and by June 12, 1928 the tumor had disappeared to palpation. The patient was discharged and on July 19, 1928 returned. There was no palpable tumor at that time. He was given another course of X-ray. October 15, 1928 he was readmitted a second time. He had gained 18 pounds and had returned to regular work. On February 18, 1929 he returned the third time. He had lost 40 pounds in weight, much of his strength and had pains in the chest. The pelvis contained a large mass of tumor and it was felt that there were also metastases to the right chest. Fluid accumulated in the chest, requiring tapping. He became very short of breath and died June 23, 1929.

*Postmortem Examination:* The right lung, liver and diaphragm were agglutinated by a very cellular, friable tumor. They were removed together and the combined weight was 3,400 gm. There was a large mass in the dome of the liver which continued through the diaphragm and involved most of the lower lobe of the right lung. The liver contained several additional nodules. The inferior vena cava, posterior to the liver, was surrounded by tumor but it was not invaded. The right kidney was markedly, and the left slightly, hydronephrotic. The ureter of the right kidney was obstructed by a scar. Both ureters were surrounded by tumor and there was tumor in the pelvis. One testicle had a nodule in it 2 by 3 cm. which upon section showed nothing but sclerosis.

*Histological Examination:* The histology of the tumor was in every way similar to the large, round cell undifferentiated carcinoma of the testicle (Fig. 7).

CASE 9. *History:* G. H. (59861), a white girl 3 years old, entered the hospital. She did not walk until two years of age and has never more than hobbled about. A month ago she fell down stairs and has walked but little since then. She cries

ally round, but at times oblong cells containing distinct nucleoli were present. There was practically no stroma. At times a pseudorosette was seen and a diagnosis of neuroblastoma was made. Death occurred Oct. 11, 1928.

*Postmortem Examination:* The left kidney was absent, but a smooth mass remained in the retroperitoneal region on the left side which extended from the diaphragm to the pelvis. It measured 18 by 8.5 cm., had a well defined capsule and was slightly adherent to the descending colon. Its cut surface was white, firm and roughly lobulated. The mediastinal glands were involved with the same type of tissue and both lungs showed nodules measuring 2 mm. to 3 cm. in diameter. The liver contained a single metastasis. The left adrenal was absent.

*Histological Examination:* The histological findings of the neoplastic mass would pass very well for a so-called embryoma of ovary or testicle. This mass was interpreted as primary and the lesion in the kidney was considered metastatic.

**CASE 8. History:** C. R. (C 3247), a white male 39 years old, entered the hospital May 19, 1928, after four days of anuria. He had been well until April, 1928, when his legs from the groin down began to swell and became sore. This lasted one and a half weeks, when the swelling disappeared and the pain subsided with it. In the latter part of April the patient was troubled with nausea and a foul taste in the mouth. For about two weeks previous to admission the patient occasionally vomited very bitter green material. The edema reappeared during the last two weeks. He was able to pass urine normally until May 16 when he passed a very small amount. May 17 he passed only a few drops and anuria developed. A tumor was then found in the abdomen by a physician. There had been a 10 pound loss of weight.

On physical examination the patient lay in bed comfortably but appeared drowsy. The breath had a definite urinous odor. Blood pressure 190/90. The abdomen was rounded and the whole epigastrium above the umbilicus was filled with a hard, nodular, slightly tender, fixed tumor which extended to the rib angle on either side. Anuria was present.

The hemoglobin was 60 per cent, red blood cells 3,900,000 and white cells 8,900. Specimen catheterized from the left kidney pelvis showed a trace of albumin.

The patient was cystoscoped immediately upon admission to the hospital. The bladder was negative. The ureteral catheter could be passed to the pelvis of the left ureter, even though an obstruction was encountered 18 cm. from the ureteral orifice. An obstruction was encountered two-thirds of the way up the right ureter which the catheter would not pass. There were 120 cc. of urine in the left kidney pelvis. The catheter was left in place to drain off the secreted urine. 1 cc. of phenolsulphonephthalein given intravenously did not appear in the urine for forty minutes. Pyelograms showed the left pelvis distinctly hydro-nephrotic with the calyces clubbed and obliterated. The ureters as outlined swung anteriorly, instead of traveling along the posterior parietes. The right

retroperitoneal mass was primary or secondary to the kidney tumor. Its large size and position, with complete replacement of the spleen, would speak strongly for a primary tumor.

*CASE 11. History:* L. T. (C 1737), a negress, 70 years of age, was in usual health until October, 1927, when she first noted severe shooting pains in the lower back and up and down both legs. Later there was numbness and a prickling sensation. The legs then became weak and on March 18, 1928, she was unable to walk. During this time the patient lost 50 pounds in weight.

Extending from the left kidney region forward, and slightly to the right of the midline, was a tumor filling the whole of the left abdomen. It was fixed posteriorly and moved but little by palpation and respiration. The mass was hard, apparently solid and slightly tender. Strength of the various flexors and extensors of the lower extremities varied from 5 to 25 per cent. Sensations other than pain and thermal were estimated from 30 to 75 per cent. Pain and thermal were normal.

The hemoglobin was 40 per cent, red blood cells 2,500,000. On March 13, 1928 there were 6,200 white blood cells, while on March 27, 1928 they were estimated to be 24,600. Gastric analysis was negative. Rectal examination revealed no pathology. Urine was negative and the stool contained occult blood.

X-ray therapy was given for an inoperable retroperitoneal tumor and it decreased one-third in size. The patient then developed fever, and a leucocytosis of 24,000 and died on March 29, 1928.

*Postmortem Examination:* There was edema of the lower extremities, more marked on the left. A large mass was found in the left abdomen. The left ureter passed for the most part anterior to the mass, but it was constricted, causing hydronephrosis. The tumor was for the most part in the left lower quadrant. The left psoas muscle, nerves, ureter, ascending colon and the ileum were invaded, but no abdominal organ was primarily involved. There were no distant metastases.

*Histological Examination:* The tumor was a definite adenocarcinoma with eccentric lumina, a type of tumor frequently seen in the ovary.

*CASE 12. History:* L. A. (E 2426), a white female 37 years of age, complained of severe pain in the left upper quadrant in August, 1928. The pain was usually dull, but occasionally it was distinctly crampy and radiated down the back and left flank. She was hospitalized from July 7, 1929 to February, 1930 because of this, and opiates have since been regularly required. In May, 1929, the patient noted a lump in the left upper quadrant which was near the midline. It did not respond to X-ray therapy. The Wassermann was found to be positive and 29 "arm shots" and 35 "hip shots" were given without result. Potassium iodide was taken until three months ago. Shortly after the appearance of the abdominal mass, another mass was found in the left axilla. The abdomen was scaphoid except for fullness in the left upper quadrant, beneath which was a

out in the night from pain. There was evidence of healed rachitis. Marked fullness in the back extended from the mid-dorsal region to the fifth lumbar vertebra. When standing, the patient bent forward in a curious manner.

She was operated upon July 7, 1924. Incision was made just below the twelfth rib. After separating the quadratus muscle, a pinkish tissue was encountered from which a biopsy was made. The tissue was made up of large, round cells with little stroma. The nuclei varied widely in their ability to absorb dye. Lymphocytes were scattered throughout. Death occurred September 9, 1924.

*Postmortem Examination:* There was generalized edema and the veins were prominent over the abdomen and about the umbilicus. The prominence of the left lower back measured 10 cm. in diameter. The appendix was bound down by firm, fibrous adhesions. The abdominal contents were pushed aside and to the right by a large retroperitoneal neoplastic mass which measured 15 by 7 cm. in diameter. The left kidney was pushed up, placed on the upper surface of the tumor, but definitely distinct from it. There was a great deal of necrosis but the viable portions were white, granular and friable. The tumor caused pressure atrophy of the tissue about the spinal column and the finger could be passed around the vertebra. The inferior vena cava and right auricle contained tumor. The lung showed metastases 1 cm. in diameter.

*Histological Examination:* Histological study revealed very large round cells with practically no stroma. Lymphoid cells were scattered throughout.

*CASE 10. History:* The patient was a female child 2 years of age, who had a large mass in the abdomen. At operation the mass was distinctly retroperitoneal, but it could not be demonstrated with certainty whether it arose from an organ or not. A piece of tissue 3 cm. in diameter was removed which was gray, uniform in structure and friable.

*Histological Examination:* Tubules cut in cross and longitudinal diameters supported by a stroma of round cells such as make up the stroma of uterine endometrium were revealed. Surrounding the foci of glands and stroma were elongated cells, some of which appeared to be smooth muscle cells (Fig. 8).

*Postmortem Examination.* There was a large, retroperitoneal mass extremely friable and brain-like in appearance back of the lesser peritoneal sac. The spleen could not be identified and it was thought to be completely replaced by tumor. There was a mass of tumor 8 cm. in diameter in the lower pole of the left kidney. Lung metastases were present. It was not interpreted whether or not the large

to the transverse colon. Stereoscopic pyelograms showed the left kidney to have normal contour. The pelvis and the upper part of the left ureter were slightly dilated and the ureter was pushed somewhat to the right as if the tumor were back of the kidney and ureter. The spleen was visualized and appeared normal in size. What was taken to be the tumor was at the lower pole of the left kidney. When the tumor was moved there was movement of both tumor and kidney but the displacement of the kidney in no way equaled that of the tumor (Figs. 12 and 13). The function of both kidneys was impaired.

The patient was operated upon January 16, 1929. There was a grapefruit-sized mass overlying the space between the stomach, spleen and transverse colon, the latter being pushed down. It was most intimately attached to the pancreas but the left kidney, though pushed down, was entirely unattached. The mass was removed.

On gross examination the tumor mass weighed 660 gm. It was very well encapsulated and in the capsule many large vessels were observed. A few cysts several centimeters in diameter protruded above the surface. When they were incised a bloody fluid escaped. On cross-section 90 per cent of the area was dull, red and lusterless, indicating dead tissue. The remaining 10 per cent consisted of cream-colored, granular, friable tissue which appeared viable. From these areas sections were taken.

*Histological Examination:* Sections showed cells arranged in cords with large, thin-walled blood vessels between. There were whorls of these cells which called to mind a renal glomerulus. The individual cell did not contain much lipoid material. It was felt at the time, from the position and histology of the tumor, that it was an adrenal carcinoma (Fig. 14).

*Subsequent Course:* The patient had a satisfactory postoperative course and was discharged on February 12, 1929. He was given three X-ray treatments over the area from which the tumor was removed. On December 30, 1930, approximately two years after the operation, he was well.

CASE 14. *History:* M. F. (E 1661), a white female, 51 years of age, had had intermittent pain in the upper abdomen for two years. The first attack was in the left upper quadrant, but all attacks since have been in the right abdomen. After these attacks large amounts of urine were passed which did not contain pus, blood or stones. There have been five such attacks in all. There has been no jaundice or clay-colored stools. There was slight rigidity in the epigastrium and 5 cm. above the umbilicus a point of tenderness was encountered. The palpable mass which was 10 cm. in diameter and slightly to the right of the umbilicus was movable and markedly tender. It was definitely distinct from the gall-bladder.

hard, irregular but smooth mass. It extended 8 cm. below the xyphoid on expiration, 12 cm. on inspiration, and the upper limit disappeared under the left costal margin. There was firmness posteriorly over the region opposite the tumor. The left axilla contained a walnut-sized node which was hard, quite freely movable and not tender.

The hemoglobin was 104 per cent, red blood cells 4,680,000 and white blood cells 10,100. The urine revealed nothing abnormal. The Wassermann was 3 plus. Renal function normal.

The gastro-intestinal canal was negative on roentgenological examination except that the stomach was somewhat moulded by a mass in the left upper quadrant. Pyelography revealed a very opaque tumor which stereoscoped anteriorly to the left kidney. The contour of both kidneys and pelvis was normal (Fig. 9).

At operation a mass 4.5 by 2.5 by 1.5 cm. was obtained from the axilla. It was made up of three separate but very firm nodules. The largest nodule was gray and granular and contained mucoid material. Encircling this nodule was a mass containing much gritty material, and the third nodule was completely calcified except for being divided into lobules by white, fibrous tissue.

*Histological Examination:* The gray tissue was epithelial, the cells arranged in acini with eccentric lumina. The calcified tissue was made up of epithelial rete structures which were supported by embryonic-appearing connective tissue. In this stroma calcified masses were found which resembled corpora amylacea and tended to pass into the lumina of the rete structures (Figs. 10 and 11).

CASE 13. *History:* O. R. (D 232), a healthy appearing white male of 48 years, noted a mass in the left upper quadrant in December, 1928. An attack of pain in the left lower back in November, 1928 is the only symptom that could be elicited from the patient, which might have had a bearing on the condition. The important findings in the physical examination were limited to the abdomen. With the patient in the recumbent position, the mass extended from a point midway between the umbilicus and symphysis up under the costal margin. The upper pole could be grasped on deep inspiration. Laterally, the tumor extended from the midline to the anterior axillary line. The contour felt lobular and the tumor mass was not tender. Back of the tumor under the costal margin a rounded mass thought to be kidney was felt. Splenic dullness seemed normal. A tympanic colon could not be demonstrated over the mass. The movement of the left costal margin was limited and the tumor moved with the margin. When the patient stood erect the tumor dropped practically to the symphysis and when the patient rolled on the right side, it fell well to the right of the midline.

The urine contained 8 pus cells per high-power field. The Wassermann was negative. Stool examination showed a 1 plus test for occult blood. There were 8,650 white blood cells and 4,970,000 red blood cells. Differential count revealed no pathological cells or abnormal percentages. The hemoglobin was 85 per cent.

Fluoroscopic and roentgenological examinations of the colon suggested no intrinsic bowel pathology, but did suggest a retroperitoneal tumor, posterior

tive and produced pain in the left flank. Temperature varied from 98° to 102.8° F, pulse from 84 to 134 and respiration from 20 to 32. Blood pressure was 125/85. The left chest was tapped for 550 cc. of turbid fluid which showed no organisms. The hemoglobin was 63 per cent and the white blood cells 13,600. The Wassermann was negative. Five urine examinations revealed no pathology. There were numerous right ventricular extra-systoles.

Roentgenological examination showed the left lower half of the chest opaque and the excursion of the right diaphragm was limited. Numerous tumorous opacities were scattered throughout the upper part of the left lung and the entire right lung. The heart and the mediastinum were displaced to the right. Some of the lesions were thought to be metastatic neoplasm. Barium enema filled a normal colon. There was a large mass of osseous density in the left upper quadrant which did not move freely with respiration. Pyclograms showed a normal kidney contour and a normal pelvis configuration. The mass seemed quite definitely distinct from the kidney (Fig. 17). Death occurred Nov. 8, 1929.

*Postmortem Examination:* Both lungs were studded with grayish, firm nodules. The larger masses were soft in their central portions and were miliary-sized to 3 cm. in diameter. The hilum nodes were replaced and enlarged by tumor. The liver revealed disseminated nodules of neoplasm which varied from miliary-sized to 5 cm. in diameter. The tumor mass measured 17 by 12 by 18 cm. and was distinct from the kidney. In the central portion of the upper pole of the tumor was a calcified mass which measured 6 by 2 by 2 cm. There was considerable necrosis. The left kidney was atrophic and weighted 90 gm., and the right kidney weighed 130 gm.

*Histological Examination:* The tumor showed extensive bone formation. The cancellous spaces were filled with round tumor cells. The part of the tumor not containing bone was made up of cords of cells which appeared somewhat similar to adrenal cells. They were rather small, however, and contained no free lipid (Figs. 18 and 19).

CASE 16. *History:* W. T. (E 6888), a white male 68 years of age, entered the hospital August 27, 1930, complaining of frequency (every twenty minutes day and night). Thirteen weeks previously the patient strained himself while lifting a heavy load. At this time he felt something give way in the groin. Soon afterward the scrotum became swollen and has since been tapped nine times, only to fill up quickly again. At this time frequency began which was followed by severe terminal tenesmus. He has had constant suprapubic pain, associated with intermittent excruciating pain which radiated from the left flank to the penis. Three of these attacks during the last thirteen weeks required morphine for relief. He has recently had severe dyspnea and palpitation on exertion.

Physical examination revealed senility and cardiac failure. Blood pressure 195/104. The hemoglobin was 75 per cent, red blood cells 4,000,000 and white blood cells 7,400. The Wassermann and Kahn tests were negative. The blood urea nitrogen was 40 mg.

The hemoglobin was 70 per cent and the white blood cells were 5,900. There was only slight acid in the gastric juice after histamine administration. Enzyme action of the duodenal juice could not be said to be abnormal. The content contained a considerable amount of bile. Wassermann and Kahn tests were negative. The Graham Cole test showed the gall-bladder outline but concentration of dye was estimated 50 per cent normal.

Roentgenological examination of the gastro-intestinal tract showed the duodenum pushed up by a mass immediately before or behind it. Pyelograms showed the kidney outlines and contour normal, with a mass of different density mesial to the kidney. At operation there was a small amount of clear fluid in the peritoneal cavity. No tumor of intra-abdominal organs was found on exploration. A tumor lay to the right of the vertebrae at the level of the head of the pancreas, the main mass of which was distinctly retroperitoneal. It was lobulated, gray, granular, friable tissue with a flat surface. There was a small nodule where the mass reflected onto the duodenum. It was considered inoperable and a biopsy was taken which consisted of a piece of tissue 2.5 by 1.5 by 1 cm. It was gray, granular, friable and lobulated, with little stroma.

*Histological Examination:* Parts of the tumor were indistinguishable from the so-called adrenal cell carcinoma of the kidney, while other parts were distinctly alveolar. The cytoplasm of the latter cells contained no visible lipid material. Still other areas were made up of large, round, embryonic cells (Figs. 15 and 16).

**CASE 15. History:** L. S. (D 6964), a white female 33 years old, had a knife-like pain in the left thigh and anterior groin since June, 1927. November 5, 1928 an ovarian cyst the size of a cocoanut was removed, but no histological examination was made. Since January, 1929 there has been a cough with hemoptysis. January 25, 1929, the patient experienced a sudden pain in the back which traveled to the inguinal region and down the inner side of the left thigh. It lasted fifteen to thirty minutes, and four hours later there was a chill and fever with marked diaphoresis. Similar pains were frequent during the week following. Since September 1, 1929, there has been cough with production of foamy yellow-white sputum, considerable pain in the left chest and dyspnea. 250 cc. of straw-colored fluid were removed which caused an increase in the discomfort. September 25, 1929, pain in the right chest required strapping.

On physical examination a well developed and well nourished, dyspneic patient, propped up in bed, was observed. Respirations were rendered shallow by pain. The left costal margin was immobile and the right moved but slightly. The upper left chest was tympanitic and the base was flat. Breath sounds and voice sounds were increased over the left upper chest while the lower chest was silent. The right back was dull to percussion and the left was flat. Every third heart beat was a right ventricular extra-systole. Two abdominal scars from an appendectomy and oöphorectomy were found. The left upper quadrant resisted deep palpation. The left costal margin, the anterior axillary line and a point halfway between the xiphoid and the umbilicus outlined a mass in the left upper quadrant. Respiration caused slight movement of the mass. It moved more, however, during palpation of the left back. There was tenderness with muscle spasm in the left upper quadrant and the mass seemed attached to, or firmly pressed against, the left costal margin. The Kernig test was posi-



## HISTOGENESIS AND CLINICAL CONSIDERATIONS

Two points arise when tumors come up for consideration. One, the question of histogenesis, is chiefly of academic interest. The recompense here must, for the most part, come from the satisfaction and security which goes with understanding. This question is based on fragments of truth which were derived from interval observations of a continuous embryological process. Gaps are left which must be filled in by interpretation. Application of these embryological processes exposes even wider and more frequent gaps in our knowledge of histogenesis of tumors. The other question of how to recognize tumors and what to expect of them with and without various types of interference is the practical point. Both the pathologist and the clinician are interested in this latter consideration.

Histogenesis is a much disputed question because it is largely based upon morphology. Cells do not always reproduce their kind accurately and the dispute wages about the point of what metaplastic potency may be imputed to a certain cell at a given stage of embryological differentiation. Some authors are very strict in the degree that a cell may change in form or function even within the same germ layer. Others seem to think that, in rare instances, a cell of one germ layer may mutate into the cell of another germ layer. Again, metaplasia, both progressive and retrogressive, is quite generally assumed to have a wider range in neoplasms than in injury and repair lesions. We do not wish to submerge ourselves in this abyss of controversy. We propose to accept the quite widely taught histogenetic explanations for the tumors arising within the adult genito-urinary organs and apply them to explain tumors which are unattached but of similar histopathology. In a few instances, histopathology, anatomy, embryology and comparative embryology have led us to suggest another explanation for the histogenesis than the one commonly accepted. Finally, we will compare and endeavor to correlate the histogenetic explanations offered for tumors attached and unattached to adult organs when a different histogenesis is offered for each.

Table IV will be of material aid in guiding us in this consideration.

*Histogenesis:* Histogenesis of simple cysts, with or without hair, may concern any one of several type cells. During embryonic life the genito-urinary structures and the ectoderm are practically in appo-

A cystogram showed a tumor on the right side extending from the fundus to the base of the bladder. Cystoscopy revealed infection of the bladder with a large sessile tumor. X-ray examination of the chest suggested carcinomatous metastases. X-ray treatment was applied with no result. On September 23, 1930, the patient developed phlebitis of the right leg. Two days later there was a chill followed by temperature of  $102^{\circ}$  F, and leucocytosis of 16,900. His general condition became gradually worse and death occurred on December 27, 1930, following shortly after a right hemiplegia.

*Postmortem Examination:* The mediastinal lymph nodes were enlarged by gray, granular, neoplastic tissue. Similar tissue extended along the bronchi. The head of the pancreas lay over a large, retroperitoneal mass the center of which was at the level of the renal veins. The tumor extended from the diaphragm to the bladder, surrounded and partially constricted the aorta and followed along the calyces to replace about half of the left kidney. The right ureter and pelvis were dilated and surrounded by tumor and the bladder wall was invaded.

*Histological Examination:* Renal tubules were very accurately reduplicated (Fig. 20).

CASE 17. *History:* N. K. (E 2084), a white male 65 years old, entered the hospital complaining of pain in the lower abdomen and back, vomiting, poor appetite and loss of weight. Pain was first noted throughout the lower abdomen in September, 1929. January, 1930, pain developed in the left testicle and on March 10, 1930, a small irregular lump appeared at the upper pole of the right testicle. The patient had lost 25 pounds during the last three months.

Physical examination revealed a large mass in the left upper quadrant which descended slightly with respiration. The urine was negative. The hemoglobin was 75 per cent, red blood cells 4,000,000, and the white blood cells 8,400. The blood urea nitrogen was 25 mg., uric acid 6 mg., and creatinine 1 mg.

The tumor was found retroperitoneally in the left upper quadrant. An abscess containing 15 cc. of pus was opened in the region of the fundus of the stomach. On March 29, 1930 the patient began to raise purulent sputum, became increasingly weaker and emaciated. Death occurred April 13, 1930.

*Postmortem Examination:* There was a large, retroperitoneal mass 15 by 12 by 8 cm. which completely surrounded the left kidney, the lower pole of which had been somewhat replaced by tumor. The left kidney showed hydronephrosis. There were neoplastic implants over the peritoneum, small metastases to both lungs and a single small metastasis to the liver. The right testicle contained a small hydrocele. There was a staphylococcic septicopyemia.

*Histological Examination:* The histology was that of a fibrosarcoma. It resembled quite closely fibroblastic neoplasms of the pleura, synovial surface and meninges.

sition. When muscles and vertebrae form, the genito-urinary structures are pushed forward. At this time bits of ectoderm may be carried forward with the genito-urinary apparatus. This would give an explanation for simple dermoids in such organs as kidney, ovary and testicle, as well as for simple dermoids and epithelial cysts not attached to these adult organs. Again, we have epithelial structures extending from the fourth cervical vertebra to the pelvis which may not completely degenerate and which may give rise to epithelial cysts. We do not believe that it is assigning too much progressive metaplastic potency to these cells in considering that they may account for some of the simple dermoid cysts.

The solid and dermoid teratomas are quite generally conceded to arise from totipotent sex cells when found in the ovary or testis. We found no solid teratomatous masses, but dermoid teratomas are found not only in our series, but also in those described in the literature. They are quite similar to dermoid teratomas of the adult sex glands and we assign to them an aberrant sex cell histogenesis.

When one cell in a teratoma grows with rapidity, it may metastasize and reproduce a simple tumor of the type cell which began rapid proliferation. It seems that the small, well formed teratoma first grows and then has a quiescent period, after which some particular element in that small teratoma again takes on growth and metastasizing tendencies. In this manner chorio-epitheliomas, embryomas, voluntary muscle and smooth muscle tumors from sex cells are explained when the tumors arise within the genital organs. Commonly, small teratomas of the testicle have been found which have given rise to a massive metastasis to the retroperitoneal structures. We have noted a well developed dermoid teratoma of the ovary which was about 1 cm. in diameter. It was still contained within a follicular cyst. These small teratomatous masses are not frequently found in malignant retroperitoneal tumors of the urogenital apparatus. They may be lost in the massive tumor or replaced by the rapidly growing, more vigorous malignant cell. We can see no objection to assigning sex cell histogenesis to similar embryonal tumors, *viz.*, the so-called embryomas, dermoid teratomas, chorio-epitheliomas, and certain smooth and striated muscle tumors, when they are found not attached to the sex glands.

Here we should mention again the disputed origin of the sex cell. If we accept the segregation of the sex cells during the morula <sup>2,3</sup>

TABLE IV  
*Tumors of the Urogenital Apparatus*  
 (A) *From Nephrogenous Tissue with Inclusions*

Histology	Attached	Unattached	Histogenesis
Adrenal-like cells, glomeruli, bone, tubules and alveoli	Embryonic renal cell tumors	This series In the literature	Mesonephros
Cords, adrenal cells	Adrenal cell tumors	In the literature	Adrenal cell
Adult renal cells, adult renal tubules	Adenoma, adenocarcinoma	This series	Adult renal cell
Glomeruli, rich stroma, tubules	Embryonal adenosarcoma	In the literature (?)	Renal blastema
Smooth muscle	Leiomyoma, leiomyosarcoma	In the literature	Müllerian tissue(?)
Fibroblasts	Fibroma, fibrosarcoma	In the literature	Mesothelium ?
Fibrous cartilagenous fat and bone	Mixed tumors	In the literature	Mesothelium ?
Epithelial cyst	Epithelial cyst	This series In the literature	?
Syncytial and Langhans cells	Chorio-epithelioma	This series In the literature	Sex cell

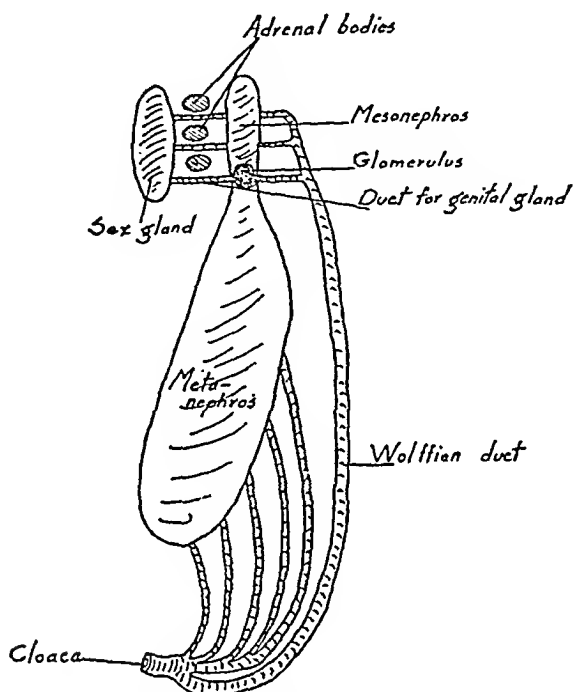
(B) *Genital Tissue with Appendages and Inclusions*

Syncytial and Langhans cells in cords	Chorio-epithelioma	This series In the literature	Sex cell
Large round cell	Embryoma	This series	Sex cell
Glandular arrangement, eccentric acini, calcareous deposit	Adenocarcinoma	This series	Rete cells
Solid, teratomatous	Teratoma	None	Sex cell
Cystic, dermoid teratoma	Dermoid cyst	This series In the literature	Sex cell
Epithelial cyst	Epithelial cyst	This series In the literature	?
Alveoli, tubules, uterine mucosa stroma, smooth-muscle	Endometrioma, adenosarcoma	This series	Embryonic Müllerian tissue
Adult endometrium	Endometrioma	In the literature	Adult Müllerian tissue
Cervical epidermoid carcinoma	Epidermoid carcinoma	None	Adult Müllerian tissue
Smooth muscle	Leiomyoma, leiomyosarcoma	In the literature	Adult Müllerian tissue
Striated muscle	Rhabdomyoma, rhabdomyosarcoma	None	Sex cell (?)
Cystadenoma	Cystadenoma, pseudomucinous	In the literature	Mesonephros
Cystadenoma	Cystadenoma, serous	In the literature	Mesonephros
Fibroblasts	Fibroma, fibrosarcoma	This series In the literature	Mesothelium?

Drawing II) the former explanation is true. Either explanation would indicate the close embryological relationship of these two structures. The metanephros arises from a continuation of the nephrogenic cord caudad to the mesonephros. It is contiguous then with the mesonephros. For these reasons it will be well to consider the histogenesis of these tumors as members of the same family.

Before the time of Grawitz, the small yellow, and even the more rapidly growing large yellowish, tumors of kidney were considered tumors of renal cell origin. The writings of Grawitz so nearly convinced the medical world, that it was infrequent that an epithelial neoplasm of the kidney was diagnosed as anything but an adrenal cell tumor. Legitimate doubt again arose because of the large number of renal tumors which had alveolar arrangement throughout, and a portion of still others was distinctly alveolar. It is true that the cells were, at times, similar and frequently resembled adrenal cells, but this distinct alveolar arrangement was not easily assigned to adrenal cells. The explanation of origin from renal tubules which were arrested in development now holds sway. Our cases which resembled adrenal cells, but in which glomeruli, bone and alveolar structures were found, developed high in the abdomen independently of the kidney. They developed above the level of the metanephric portion of the nephrogenous cord and from a portion which would correspond to the site of origin of the mesonephros. Embryologically, the mesonephros is more closely related to the adrenal than is the metanephros. Might not the adrenal-like masses found near the epididymis and the masses of adrenal-like tissue found near the hilum of the ovary of infants, as described by Marchand, be in no inconsiderable number of instances remnants of the cross tubules of the mesonephros? It would seem more logical to conclude that they were fragmented pieces of the mesonephros which had been torn away when the testis or ovary began its descent, than to suppose that they came from a more mesially situated adrenal. Is it not likely that mesonephric remnants may easily be included in the metanephros since these tissues are contiguous and may even overlap? Lastly, some of the unattached tumors which resembled the adrenal in structure, but where the adrenal was uninvolved, were high in the abdomen and had a distinctly tubular structure. Individuals who feel that this type of tumor is not of mesonephric origin have these observations to explain.

stage of the embryo, which is the more widely accepted theory, we must permit a wider distribution than if the sex cell developed after the embryo took on its definitive morphology,<sup>4</sup> as provided for in the second theory. It is obvious that the cell may get lost in its migration to the dorsal mesenchyme, or that some cells may be carried to various parts of the body when the embryo takes on its final form.



DIAGRAMMATIC DRAWING II  
Urogenital system of salamander

We may, in this way, account for chorio-epitheliomas in such places as the liver, mediastinum and pineal gland.

We may dismiss the pronephros by stating that we have found no information about it, unless this structure accounts for some of the simple dermoids found in the mediastinum. There also seems to be no information on unattached tumors from the very embryonic renal blastema. Solid tumors of the mesonephros and the adrenal are either from the same ontogenic<sup>5</sup> tissue or not far removed in phylogeny.<sup>6</sup> It is believed by some that the adrenal develops from the cross tubules of the mesonephros, while others are equally certain that the adrenal arises from a separate invagination of the mesenchyme by celomic epithelium.<sup>6</sup> In the salamander (see Diagrammatic

No explanation has been given for these types of tumor when they occur in the ovary.

The Müllerian apparatus will now be considered. Conservative pathologists have not been satisfied with the explanation of Sampson for all endometrial-like tumors occurring in aberrant locations. The theories of histogenesis are well summarized by Clarke.<sup>1</sup> When tumors of this sort occur only along the course of the embryonal Müllerian apparatus it seems more logical to believe that they had their origin from that structure, than to explain that they passed through this particular part of the peritoneum without leaving a scar. This would explain endometriomas, either glandular or epidermoid, in the rectovaginal septum and retroperitoneally. It also opens a likely explanation for the frequency of leiomyomas retroperitoneally. The tumor in Case 10 of our series resembles very closely the so-called adenosarcoma of the endometrium.

The potency of mesothelium has been appreciated since it has been established that synovial and pleural tumors produce bone, fat, fibrous tissue, angiomatous tissue and glandular tissue. We assume that this might account for many of the mixed tumors with no particular characteristics which will associate them with a more definite embryonal structure. Such tumors are found both within and without the adult genito-urinary organs.

*Clinical Facts Common to Tumors of this Series:* Clinically, there are points of general interest which pertain to all tumors of this series. Age is not a discriminating feature because they may occur throughout the life span of an individual. Frequency is also of minor importance since there has been only slightly less than one tumor reported annually in the world's literature. We might state here that the chances are not few that many are wrongly interpreted in the archives of the various clinics. The left side, and especially the left upper quadrant of the abdomen, is the most usual site for these tumors. There is no explanation found for this fact. There seems to be a natural division into solid and cystic tumors. The cystic tumors occur much more frequently in females. There is a striking difference in the results from treatment. Prognosis after removal of the pseudomucinous and serous cystadenomas, if they have not as yet invaded surrounding structures, is good. The treatment of dermoid cysts is hampered by their attachment to surrounding structures because of the inherent nature of this tumor to cause adhesions. The

It would seem that the diagnosis of adrenal cell tumor would be warranted only when the histology was adrenal-like throughout, or the tumor manifested the function of the adrenal cortex. When all is said and done, we anticipate that there will be members of this group so nearly alike that an assignment of histogenesis cannot be made with certainty. To the more definitely tubular tumors, which at times are papillary, the adult renal cell is assigned for histogenesis. We had one such case in our series of unattached tumors. It arose from the lower part of the nephrogenic cord and was first recognized as a bladder tumor because it had invaded this organ. This caudad origin in the nephrogenous cord substantiated the accepted theory of histogenesis.

The cystic tumors of the embryonic mesonephros, when they are found unattached, resemble the serous and pseudomucinous cystadenomas of the ovary. There is, at times, an attempt at glomerulus formation in the walls of the cysts. The explanation quite widely accepted is that they are remnants of the Wolffian duct and that the two types of cysts vary only in the function of the epithelial cell lining the cyst wall. Natural history of these tumors and similar tumors arising within the ovary does not warrant any further distinction. The ovarian pseudomucinous and serous cystadenomas are frequently interpreted as arising from ingrowth of the epithelium covering the surface of the ovary and are, therefore, closely related to the epithelium of the Graafian follicle. It is possible that cysts may arise in this way also, but the former explanation seems to us the more likely and the better substantiated.

Whether or not the rete structures are part of the sex gland, or a continuation of the cross tubules from the mesonephros which connect the sex gland with the Wolffian duct, is not certain. Two of our cases, we believe, are from rete structures. Roessle and Wallart<sup>7</sup> reported a case of hyperplasia of the rete structures associated with infantilism. There were nodules of adrenal-like tissue attached, which we consider were part of the contiguous cross tubules of the mesonephros. The rete structures resembled very closely the psammomatous portion of the tumor in Case 12 of our series. Both epithelial cells and arrangement, as well as the stroma, are similar. This led us to the opinion that the cell masses containing the numerous eccentric acini found in another portion of this psammomatous tumor are a more abortive attempt at the formation of rete structures.



contained psammoma bodies. The metastasis in this case was unusual in that there was a very mixed histological picture in addition to the calcification. An infected cyst will give the clinical picture of a retroperitoneal abscess which at times resembles a perinephric abscess. These tumors seem to arise deep in the retroperitoneal structures when they are not pushed forward by the adult kidney. In three of our cases there was paresis of the lower extremities, and in two of these the tumor invaded between the laminae of the vertebrae.

*Points of Practical Interest about Individual Tumors of this Series:*

A few points of interest about individual tumors may deserve separate consideration. There are many possibilities, retroperitoneally, for simple epithelial cysts. We have epithelial structures of various sorts extending from the fourth cervical vertebra, where the pronephros begins, to the pelvis. They may be lined by epithelium and may also contain hair and immature hair follicles. The capsule is usually quite thick and there are adhesions to the surrounding tissues and organs which cause difficulty in removal. The thickness of the wall, coupled with a large amount of brownish, grumous content, should lead one to suspect a preformed cyst in contradistinction to a chronic abscess, at the time of operation. Hair in the cyst content renders the diagnosis secure.

Dermoid teratomas are similar except that they contain multiple cysts with many well defined hair follicles. The contents of the cysts, in addition to being grumous, are greasy. Hair is usually abundant and coarse. Bone in the tumor, as shown in the roentgenograms and at the time of operation, is very significant. When these teratomas become infected, granulation tissue covers the walls of the various cysts and the cavities are filled with hair and puriform material. The natural history is similar to dermoid cysts of the ovary. Our case had a history of a mass in the left upper quadrant for thirty-six years. This tumor most likely arose from a sex cell as the genital fold extends from the first dorsal vertebra to the pelvis. Furthermore, if we accept the theory that sex cells are segregated in the morula stage of the embryo, we must permit a wider distribution of this tumor than is indicated by the extent of the genital fold.

The massive undifferentiated epithelial tumors (embryomas), have a natural history very similar to a metastasis from the testicle.

results from removal of the solid tumors are very disappointing. There is a ray of hope even here, since the patient in Case 13 of our series is alive and well two years after removal of the tumor. This tumor was very movable, not having mingled with the surrounding tissue. Perhaps we shall in the future be able to diagnose more tumors at this stage of development. This will be more probable if urologists will make a special point of looking for these tumors in the routine examination of the genito-urinary tract. The filling of numerous cysts by secretion in a cystadenoma markedly increases the size of the mass which is likely to attract the attention of the patient and the physician early, before the tumor has mingled with the adjacent retroperitoneal tissue. Small retroperitoneal solid tumors of like age may remain quiescent for years and not be found because of their size. Even cystic tumors have been known to metastasize after twenty-six years of quiescence. These tumors must all be considered potentially malignant. The capsule is sooner or later invaded; there is mingling with surrounding tissues, implantations on the peritoneum may result, and finally more distant metastases occur. It will be noted that this natural history is the history of similar tumors in the adult urogenital organs.

Unless metastases have taken place we have only a tumor in the abdomen, with its associated discomfort, to consider. These tumors are usually quite massive. Cystoscopy with pyelograms, followed by stereoscopic plates, has been of the greatest value. A normal pelvis and a normal kidney contour with a massive retroperitoneal tumor renders the diagnosis of a urogenital apparatus tumor very likely. The stereoscopic study gives one some idea of the relative position of the kidney and of the mass. If the tumor is movable, the question of whether it moves independently of the kidney may be of value. The injected kidney pelvis may be considered a fixed point and the tumor may be moved by palpation as shown in Figs. 12 and 13 from Case 13. Incidentally, roentgenological examination of the gastro-intestinal tract will usually show distortion from the expansive growth of the tumor. Tumors of the gastro-intestinal tract are usually not so large and globular. A cyst (lymphangiomatous, dermoid or epithelial) however, may arise between the leaves of the mesentery. Tumors of the adrenal, mesonephros, or rete structures may collect calcium in the form of psammoma bodies, amorphous calcium, or bone. The metastases in one of our cases

cells. This type of tumor is occasionally mixed with a tumor composed of masses of epithelium, each mass containing numerous eccentric acini which may be an attempt to reproduce rete structures. This type of tumor is easily recognized. Our case (Case 12) showed metastases to the axilla. These metastases showed both the adenocarcinomatous areas with psammoma bodies and epithelial nests containing numerous acini.

Tumors from the mesonephros may be cystic. Here, we refer to the so-called serous and pseudomucinous cystadenomas. The natural history of these tumors in the ovary is so well known that space is hardly warranted to present a natural history for similar unattached tumors. They may grow to weigh 20,000 gm. They may become malignant after twenty-six years of quiescence. Extremely large cystadenomas may be ruptured as a result of strain upon the abdomen. The favorable cases for surgical interference are of this group.

Leiomyomas occur retroperitoneally unattached, as well as within the kidney. They are somewhat more malignant than leiomyomas of the adult uterus, but are otherwise similar. A rather friable mixed tumor was found in the region of the left kidney which had invaded the kidney and had the histological picture of an adenosarcoma of the endometrium. Its invasiveness resembled the similar tumor occasionally encountered in the adult uterus. More benign tumors reduplicating adult endometrium are found in the rectovaginal septum, and as time goes on we predict that they will also be found retroperitoneally along the course of the Müllerian apparatus. One of us (G. H. H.) has on one occasion seen a carcinoma in the rectovaginal septum which resembled a carcinoma of the cervix. Such are the possibilities of the Müllerian apparatus.

Fibrosarcomas as well as tumors containing fibroblastic tissue, fat and cartilage, are found retroperitoneally. They usually grow to large size before metastases occur.

#### SUMMARY AND CONCLUSIONS

1. Seventeen retroperitoneal tumors which were not attached to adult urogenital organs are reported.
2. All tumors reported are similar to tumors which arise in the adult urogenital organs.

They become very large, degenerate and metastasize by invading veins. They are soft, friable tumors. Ureters are surrounded and occluded, leading to anuria, as found in one of our cases. They, at times, respond well to X-ray treatment. The tumor being of sex cell origin may be found elsewhere than along the course of the genital fold, but because of its indifferent nature it probably would not be recognized in aberrant locations.

Chorio-epitheliomas have been reported in the liver, the kidney, the mediastinum and the pineal gland. Askanazy's case of pineal chorio-epithelioma was accepted by Bonney upon examination of the material. Even though we must be cautious in diagnosing a tumor which is typical, as a chorio-epithelioma, on morphology alone, when the tumor is found in such remote places as the pineal gland we must admit that the early segregation of the sex cell permits such wide distribution. The tumor is usually soft and dull red in color. The natural history is that of chorio-epithelioma of the uterus, testis or ovary.

We have not as yet observed any solid tumors from the pronephros. The solid tumors of the mesonephros, the metanephros and the adrenal may be considered as a family. The natural history of these tumors is similar to adrenal cell carcinoma and renal cell carcinoma of the adult kidney. They are likely to be yellowish in color. Necrosis is frequently a prominent feature in them. They manifest a great tendency to invade veins and grow along them as tumor thrombi. By the time they are diagnosed they usually have metastasized. The histopathology is typical adult adrenal cortical cells in cases of adrenal cell tumors. There may or may not be adrenal medullary cells or excessive adrenal cortical function. The mesonephric tumors have adrenal-like cells. There is an alveolar arrangement of cells or there is mixed alveolar and adrenal-like histology. At times, there is an attempt at glomerulus formation. Tumors of the metanephros are likely to arise from the caudad portion of the nephrogenic cord. They may invade the bladder and kidney. The histology is, at times, very like adult renal tubules. Calcification and ossification occur more particularly in the adrenal and mesonephric tumors.

Another tumor closely related to the mesonephric tissue and the adrenal is the so-called psammoma of the ovary. Evidence has been set forth to show that this type of tumor may come from the rete

## DESCRIPTION OF PLATES

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### PLATE 141

FIG. 1. Case 1. Photomicrograph showing a thick wall of acutely inflamed granulation tissue in which many cholesterol clefts are present.  $\times 130$ .

FIG. 2. Case 3. X-ray showing large unilocular calcified cyst which stereoscoped anterior to the kidney. History of forty-six years of upper abdominal pain.

3. Retroperitoneal tumors collected from the literature integrated with the material of this paper have shown that almost all tumors which occur in adult urogenital organs may occur free along the course of development of the urogenital apparatus.

4. The concept that they arise from remnants of the urogenital apparatus is the most logical explanation of their histogenesis.

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PLATE 142

FIG. 3. Case 5. X-ray showing chorio-epitheliomatous metastases in the lung of a twenty-six year old male with a primary retroperitoneal chorio-epithelioma. See Figs. 4, 5 and 6.

FIG. 4. Case 5. Massive hemorrhagic metastases in the right lung from a large primary retroperitoneal chorio-epithelioma. See Figs. 3, 5 and 6.



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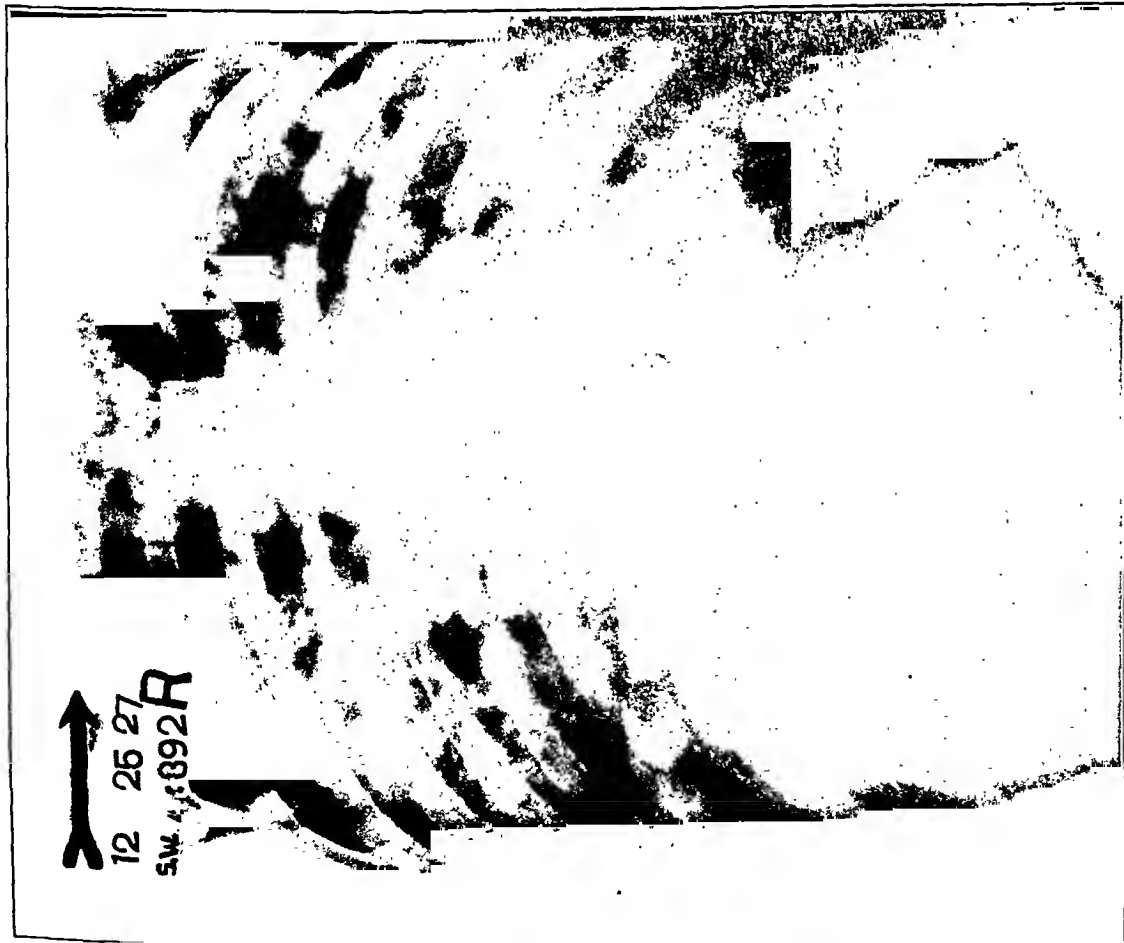
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PLATE 143

FIG. 5. Case 5. Cut section of lung showing massive hemorrhagic pulmonary metastases from the tumor shown in Figs. 3 and 4.

FIG. 6. Case 5. Photomicrograph showing typical chorio-epithelioma. See Figs. 3, 4 and 5.  $\times 130$ .



3

Hansmann and Budd



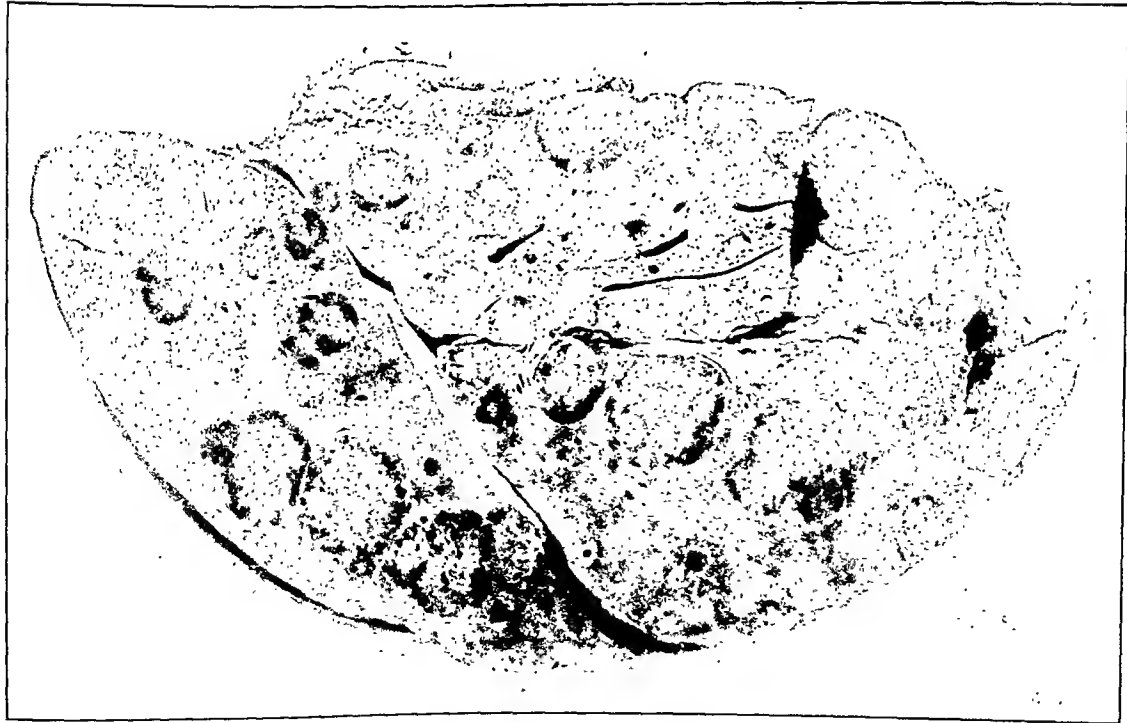
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Massive Unattached Retroperitoneal Tumors  
Massive Unattached Retroperitoneal Tumors

PLATE 144

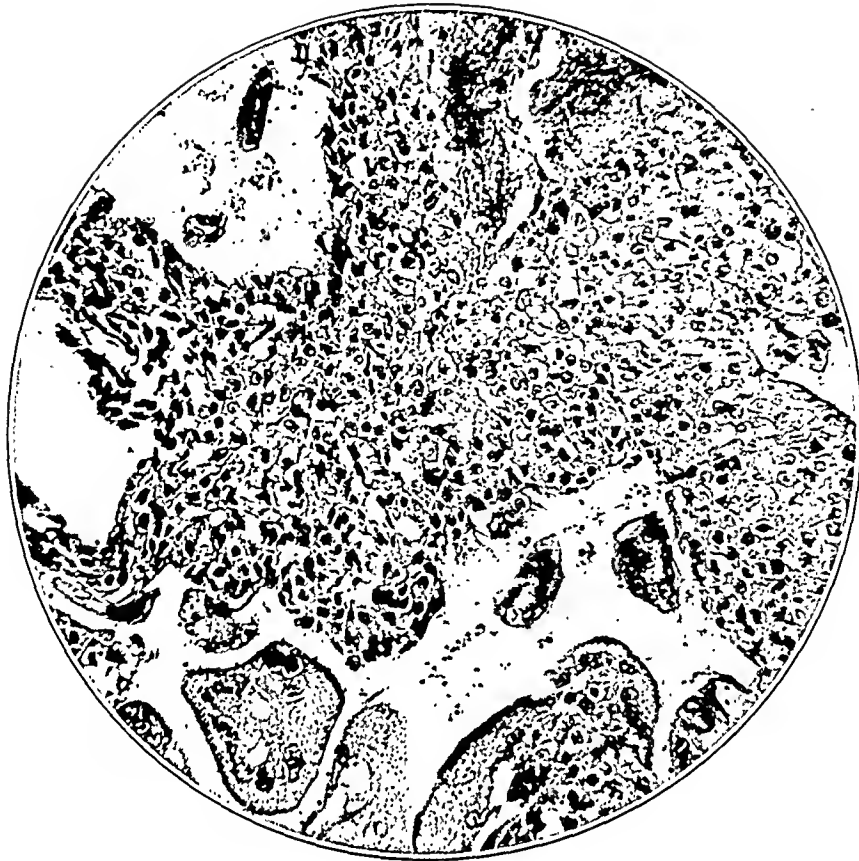
FIG. 7. Case 8. Photomicrograph showing large round cells with slight adenomatous differentiation. Similar to the so-called embryoma of the testicle.  $\times 130$ .

FIG. 8. Case 10. Tubular structures and small round cell supporting stroma with what appears to be smooth muscle at periphery. Reminiscent of uterine endometrium.  $\times 130$ .



5

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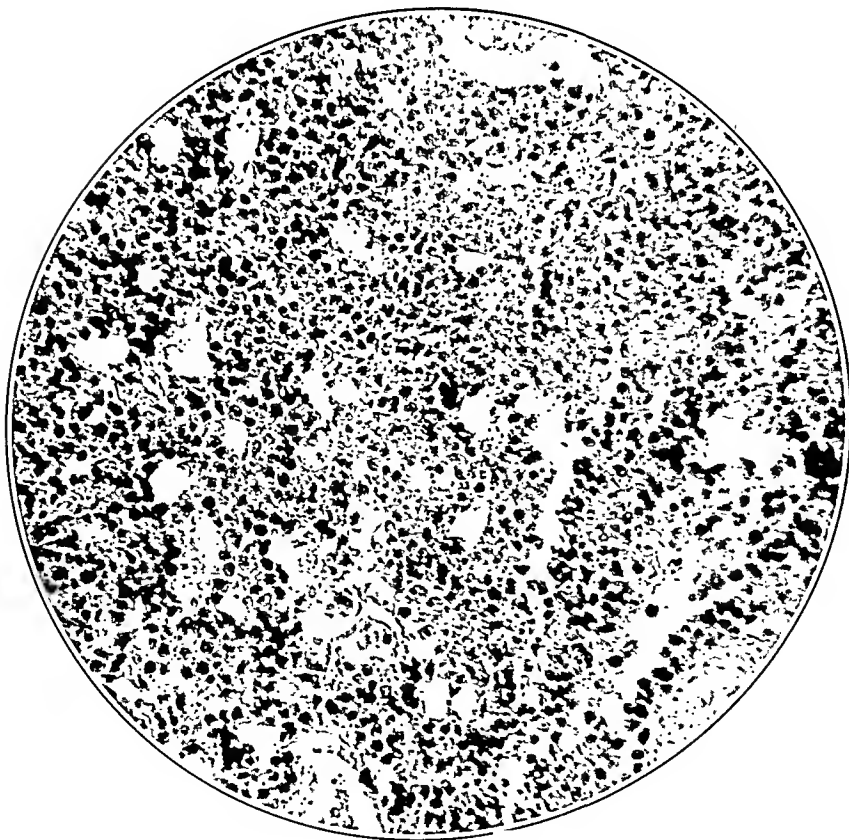
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Massive Unattached Retroperitoneal Tumors

PLATE 145

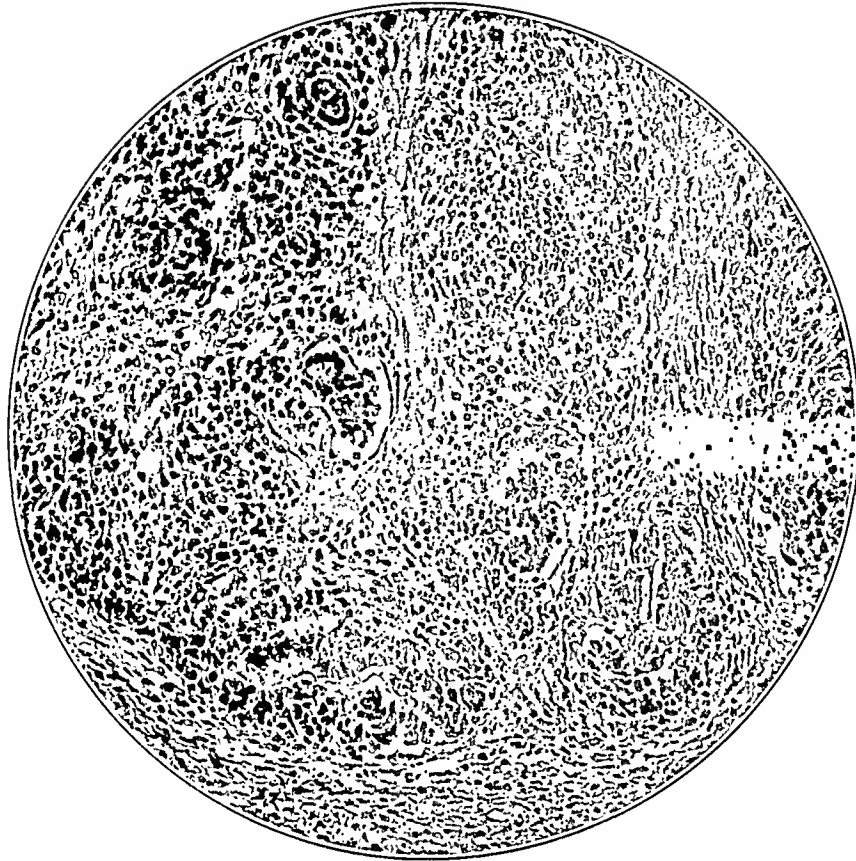
FIG. 9. Case 12. X-ray showing large calcified mass anterior to left kidney.  
See Figs. 10 and 11.

FIG. 10. Case 12. Photomicrograph showing large calcareous masses in the tumor shown in Fig. 9. Similar to Fig. 286, a carcinoma of the ovary, in Ewing's "Neoplastic Diseases." See Figs. 9 and 11.  $\times 130$ .



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8

Massive Unattached Retroperitoneal Tumors

PLATE 146

FIG. 11. Case 12. Photomicrograph showing adenocarcinoma with eccentric acini. See Figs. 9 and 10.  $\times 130$ .

FIG. 12. Case 13. X-ray showing tumor in left upper quadrant. See Figs. 13 and 14.



9

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10

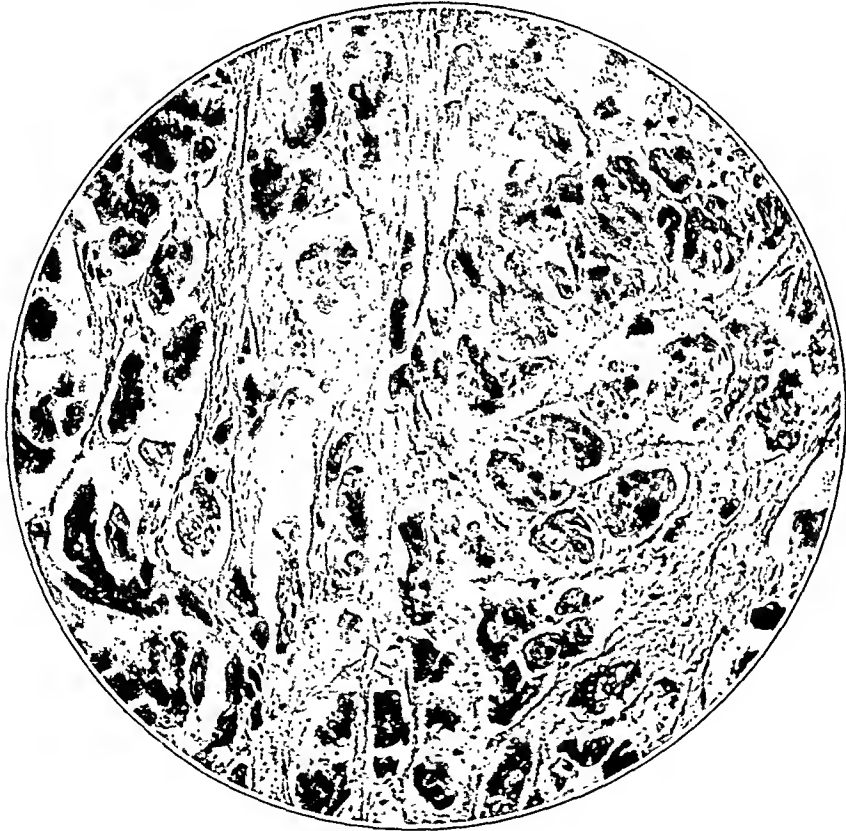
Massive Unattached Retroperitoneal Tumors



PLATE 147

FIG. 13. Case 13. X-ray showing displacement of tumor by palpation. It moves independently of the kidney. See Figs. 12 and 14.

FIG. 14. Case 13. Photomicrograph of tumor shown in Figs. 12 and 13, showing adrenal-like cells and arrangement with attempts at glomerulus formation.  $\times 130$ .



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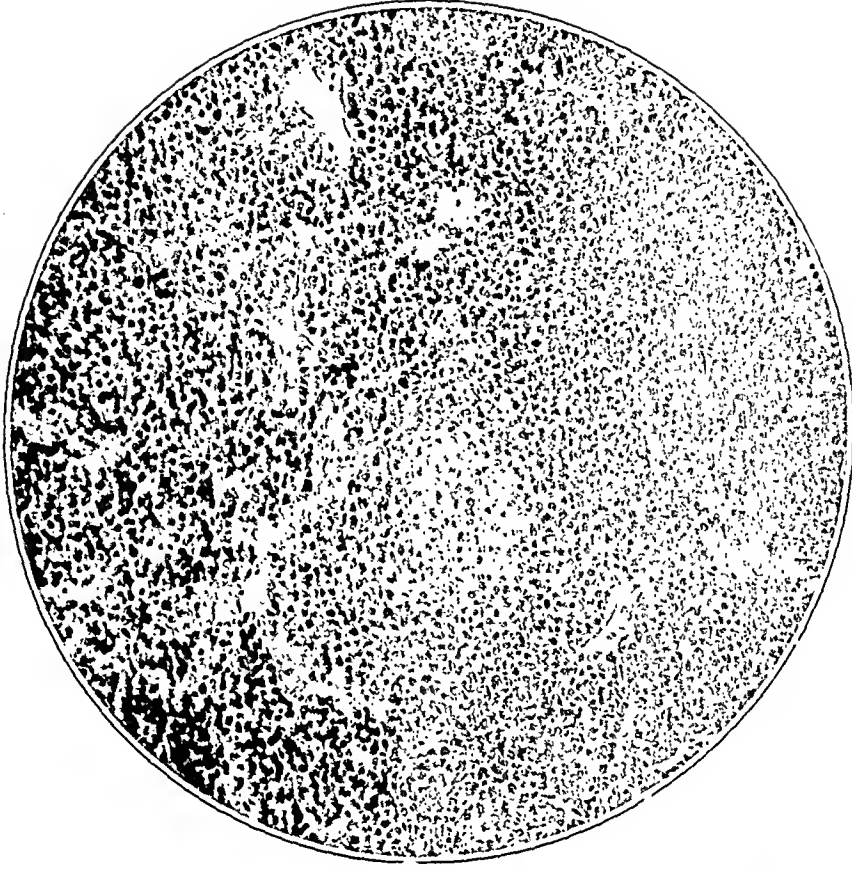


TABLE I

Differential characteristics	Papilliferous cystadenoma	Papilliferous cystadenocarcinoma	Papilliferous adenoma	Papilliferous malignant adenoma	Papilliferous carcinoma
Origin	10 in thyroid gland 17 in aberrant thyroid tissue	20 in thyroid gland 15 in aberrant thyroid	15 in thyroid gland 2 in aberrant thyroid tissue	16 in thyroid gland 3 in aberrant thyroid tissue	4 in thyroid gland
Pathogenesis	Hyperplasia of lining of a cystic adenoma	Malignant change in a papilliferous cyst-adenoma	Intra-acinar epithelial hyperplasia in a follicular adenoma	Intra-acinar hyperplasia in a malignant adenoma	Focal hyperplasia in non-tumorous thyroid gland
Gross structure	Cystic	Cystic	Solid	Solid	Solid scirrhous and small. Not encapsulated
Acini	Secondary differentiation of papilliferous epithelium	Secondary differentiation of papilliferous epithelium	Primary structural unit of tumor	Primary structural unit of tumor	Atypical
Papillae	Coarse, arborescent, macroscopic, and seat of degeneration	Coarse, arborescent, macroscopic, and seat of degeneration	Delicate and simple or complex and usually microscopic	Delicate and simple or complex and usually microscopic	Microscopic and simple
Secondary changes	Fibrosis, lymphocytic infiltration, old and recent hemorrhage, calcification	Fibrosis, lymphocytic infiltration, old and recent hemorrhage, calcification			Fibrosis, lymphocytic infiltration
Malignancy	None	Capsular invasion metastasis to regional lymph nodes	None	Blood vascular invasion, metastasis to regional lymph nodes, capsular invasion	None



13



14

cystadenomas and cystadenocarcinomas constituted a group of tumors having peculiar and characteristic growth. The term "papilliferous" as applied to tumors of the thyroid is significant only in connection with papilliferous cystadenomas and papilliferous cystadenocarcinomas.

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such tumor of the thyroid as an adenocarcinoma not arising in an adenoma. These lesions simulated tumor because of the sharp distinction between their cells and structure and those of the surrounding thyroid gland (Fig. 4). The size, shape and staining qualities of the epithelial cells, the shape of acini, the character of the papilliferous hyperplasia, the amount and disposition of stroma and the sharp delimitations of the lesions differentiated them from the usual manifestations of hypo- or hyperinvolution in glands, the seat of secondary hyperplasia. They more nearly resembled malignant than benign tumors, because of the absence of encapsulation and the apparent invasion of a densely fibrous stroma which was continuous with the interstitial tissue of the surrounding gland.

There were several reasons for considering these lesions non-tumorous. The absence of a capsule militated against the probability of benign tumor. Their small size, their failure to invade adjacent tissue and the non-existence, in a collection of over 130 malignant tumors of the thyroid, of a type of tumor which had apparently evolved from such a lesion, argued against a malignant tumor and led us to consider other pathogenetic possibilities. All four of the lesions studied were small, subcapsular and situated in glands altered by abnormal involution. In thyroids the seat of pathological involution, any and all of the features characterizing the lesions described *may* occur, and the fact that all of these changes occurred in a single isolated area was unusual but did not of necessity indicate neoplasia.

### SUMMARY

1. One hundred and two papilliferous tumors of the thyroid or of aberrant thyroid tissue were studied and classified. Twenty-eight of these were from the Institute of Pathology of Western Reserve University and seventy-four were published case reports from other sources.

2. The differential characteristics of the various types are tabulated in Table I. As indicated in the discussion of the various types of tumors these characteristics were not invariably present, but represented the features that were most common.

3. The papilliferous character of adenomas and malignant adenomas did not distinguish them from the non-papilliferous forms of those tumors so far as growth was concerned, while the papilliferous

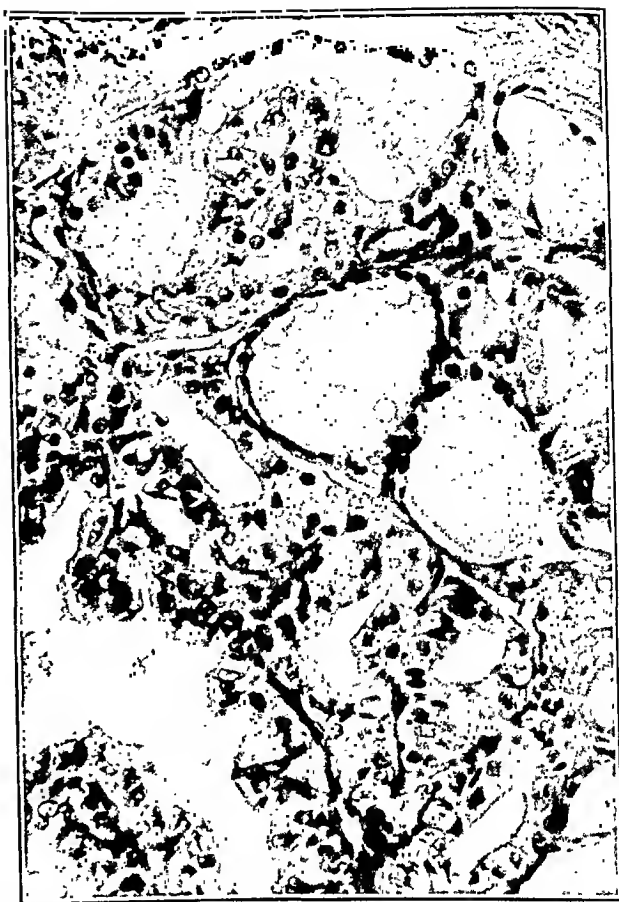
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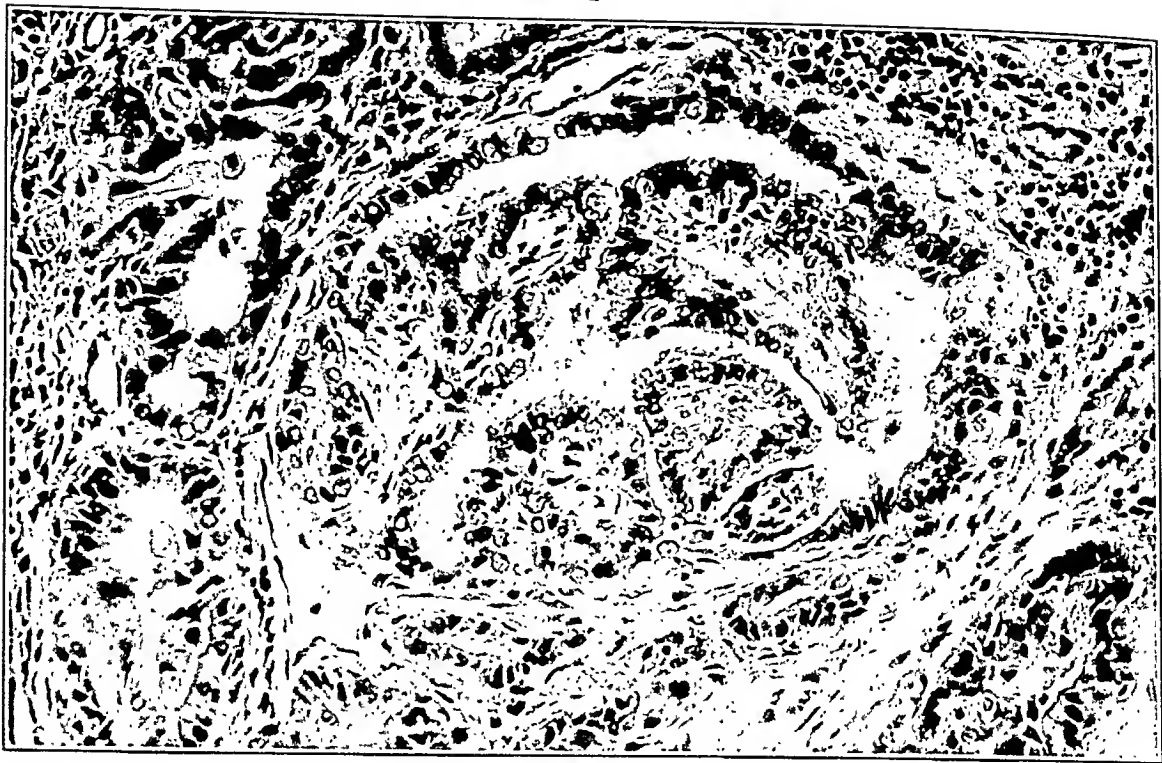
## DESCRIPTION OF PLATES

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### PLATE 151

- FIG. 1. Papilliferous cystadenoma of left lateral aberrant thyroid tissue. Actual size.
- FIG. 2. Dense hyalinized capsule of a papilliferous cystadenocarcinoma. Capsule is invaded by atypical follicles in which intrafollicular papilliferous hyperplasia of epithelium is seen.  $\times 10$ .
- FIG. 3. Portion of a papilliferous cystadenoma of lateral aberrant thyroid tissue in which original follicular structure of adenoma is not entirely replaced.  $\times 240$ .
- FIG. 4. Upper pole of one lobe of thyroid including capsule and small subcapsular papilliferous carcinoid. There is diffuse lymphocytic infiltration and proliferation of fibrous connective tissue in and around the lesion.  $\times 30$ .

7



5



6



PLATE 152

FIG. 5. Papilliferous cystadenocarcinoma of right lateral cervical aberrant thyroid gland tissue. Three-fourths actual size.

FIG. 6. Delicate arborescent papillae in a malignant adenoma.  $\times 200$ .

FIG. 7. Invasion of tissue adjacent to a papilliferous cystadenocarcinoma of lateral aberrant thyroid tissue by atypical follicles lined by pleomorphic epithelial cells which exhibit papilliferous intra-acinar proliferation. Fibrosis and lymphocytic infiltration are marked.  $\times 220$ .

# A CASE OF MYELOMA WITH UNUSUAL AMYLOID DEPOSITION \*

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A case of multiple myeloma with deposition of amyloid in the intestine and muscles of the thorax and left shoulder, together with a large "amyloid tumor" of the left shoulder joint, was reported in 1910 by Hueter.<sup>1</sup> In this instance no amyloid was found in its usual sites of deposition, namely the spleen, liver and kidneys. In reviewing the literature Hueter found only one other case of myeloma associated with amyloidosis (that of Askanaazy in 1904) in which amyloid was present in the intestine. In 1916 Claus<sup>2</sup> published a report of a case of myeloma in which amyloid degeneration was widespread, affecting the intestinal tract, mesenteric vessels, heart and striated muscles of the tongue and thorax. In addition, the substance was distributed in the blood vessels of the thyroid, pancreas, testes, liver, spleen, kidneys, adrenals and lymph glands. Calcium was present in some of the amyloid-containing areas.

Within the past few months Lubarsch,<sup>3</sup> Larsen<sup>4</sup> and Warren<sup>5</sup> have reported instances of amyloidosis of the cardiac, smooth and striated muscles, and in their papers have included brief abstracts of work previously published concerning amyloidosis of the muscular systems.

## CASE REPORT

*Clinical History:* C. P., age 38 years, male, mulatto (History No. 222791, Necropsy No. 10389) was admitted to the Presbyterian Hospital September 16, 1929.

*Chief Complaint:* Pains in the muscles, joints and bones over entire body for nine months.

*Past History:* Typhoid fever twenty-two years ago. Jaundice nineteen years ago. Amputation of right leg above the knee fifteen years ago because of elephantis.

*Present Illness:* Severe cramp-like pains began first in the thigh and leg muscles, followed a month later by pains in the wrists, hands, fingers and knee. Associated with these were stiffness and numbness of the left hand and fingers,



## AUTOPSY FINDINGS

The body is that of a markedly emaciated mulatto male with enormous shoulders in which masses are palpable. These are firm, non-movable and not sharply circumscribed. The mass in the right shoulder measures approximately 10 by 8 cm. In the left flank, filling the space between the crest of the ilium and the left costal margin, is another firm mass. On the lateral surface of the left thigh is a firm, non-movable swelling 15 cm. in length by 6 cm. in width. In the right groin, on the anteromedial aspect and immediately below Poupart's ligament, is a projecting, freely movable, oval mass 8 by 8 cm. in size, over which the skin is thick and nodular. Smaller firm masses are palpable in both antecubital fossae. Both arms are emaciated and the muscles seem small. The hands are edematous. The right leg has been amputated above the knee and the muscles of the stump are atrophied.

The peritoneal cavity contains about 75 cc. of slightly milky fluid. The left pleural cavity contains 1,000 cc. of clear, yellow fluid and the right contains 1,300 cc.

Dissection of the shoulders shows similar changes in each. The skin is not unduly adherent to the underlying tumor-like structures and there is a layer of voluntary muscle covering each mass. Section through the deltoid muscles shows that immediately beneath a thin superficial layer of muscle the entire region is occupied by a firm, lardaceous substance 3 to 4.5 cm. thick, which surrounds the shoulder joints and the heads of the humeri, extending down over them to the level of the surgical necks. These masses have invaded or replaced the regional structures so that the normal landmarks are no longer seen. On the cut surface of the "tumor" delicate and coarse bands of connective tissue separate masses of homogeneous, white, translucent material. Between the coarser bands of connective tissue are narrow clefts and spaces. The tumor-like mass extends down to the periosteum from which it can be separated easily. On opening both shoulder joints the articular surfaces appear smooth. From the glenoid fossa of the right joint a small amount of smooth, firm material, suggesting pale fat, is removed. On section of the head of the right humerus the cut surface is grayish yellow, mottled with small, white, soft masses which vary in size from 3 to 5 mm.

and stiffness of the left knee and hip. There was a gradual loss of strength and a loss of 25 to 30 pounds in weight during the last two or three months, although his appetite was greater than usual. He had polydipsia and some polyuria, the urine being either pale or milky in appearance.

*Physical Examination:* The patient was a chronically ill mulatto. The tongue showed a diffuse papillary atrophy, and the submaxillary lymph glands were enlarged. The heart was slightly enlarged to left and right, with a systolic whiff at the apex, a systolic blow in the mitral area and a systolic apical thrill. Blood pressure 120 systolic, 75 diastolic. The lungs were clear. In the abdominal wall was a firm, non-tender movable mass, approximately 10 by 6 by 6 cm. in size, lying in the oblique muscle group of the left flank. The spleen and kidneys were not palpable. The lower border of the liver lay 3 cm. below the right costal margin. The prostate was small and soft. The right leg had been amputated at the level of the mid thigh. There was an atrophy of the interscapular and erector spinae muscle groups, left deltoid, biceps and triceps, left pectoral, thenar and hypothenar groups, and the muscles of the left calf. The right deltoid, muscles of the forearms, and right pectoral appeared hypertrophied. In the left thigh a large mass was present in the abductor muscle group. On the medial aspect of the right thigh was a smaller mass.

A neurological examination reported: "Coördination, deep and superficial reflexes, sensation and cranial nerves show no impairment indicative of any localizable pathology in the central or peripheral nervous system."

Temperature 99.2 F; pulse 80; respirations 20.

*Laboratory Findings:* Hemoglobin 50 per cent; red blood cells 3,010,000; white blood cells 8,700; polymorphonuclear leucocytes 66 per cent; lymphocytes 26 per cent; basophiles 1 per cent; large mononuclear leucocytes 7 per cent. Blood urea 0.54 gm. per liter. Blood Wassermann negative. Urine: specific gravity 1.009-1.020; albumin, heavy trace; occasional red and white blood cells and granular and hyaline casts; Bence-Jones protein present. X-ray films (No. 59338) of the skeleton showed diffuse rarefaction and small areas of bone destruction in the cervical, dorsal and lumbar vertebrae, ribs, clavicles, humeri, femora, pelvis bones and skull. No spontaneous fractures were visible.

*Course of Illness:* During the patient's stay in the hospital the mass in the left thigh grew larger, his shoulders increased markedly in size and a small mass appeared in the left antecubital fossa. The Bence-Jones protein disappeared from the urine, only to reappear during the three weeks before death. Edema of both elbows, left lower extremity and sacral region developed and anemia increased, the hemoglobin on January 20 being 29 per cent, red blood cells numbering 1,660,000 and white cells 2,250, with 74 per cent polymorphonuclear leucocytes. Biopsy specimens (Surgical Pathology No. 41510, Medical Pathology No. 2054) from the left thigh and right shoulder failed to show any tumor growth. The day before death the blood pressure had fallen to 60 systolic, 40 diastolic, respirations became slow and shallow and the pulse rapid. He grew irrational, became semicomatose and died February 1, 1930, four and one-half months after admission to the hospital.

*Clinical Diagnosis:* Myeloma; leucosarcoma (?), chronic nephritis, secondary anemia.



On the anterior surface of the right lobe of the liver are fibrous adhesions. The organ weighs 1660 gm. and through the capsule appears pale brownish red in color. On section the lobular markings are small and poorly defined, and scattered over the cut surface are numerous yellow flecks 1 to 2 mm. in diameter.

The gall-bladder contains one mulberry stone.

The adrenals are of normal size but slightly firmer in consistence than usual. On section there is some pallor of their cortices.

The left kidney measures 10 by 5.5 cm. and the right 9 by 5.5 cm., each being slightly smaller than normal. The capsules strip easily. Scattered over the surfaces are numerous, slightly raised, gray elevations 1 to 2 mm. wide, which in places have become confluent. The remainder of the surface is deep reddish brown and a few cysts 1 to 4 mm. in diameter are present. On section the normal renal markings are obscured. The cortices are narrow and throughout them are innumerable grayish areas 0.5 to 2 mm. in size, while in some regions the entire cortex is gray. The pyramids are pale and streaked with red linear markings.

The bladder is distended with urine containing white flocculent masses. The bladder wall is slightly trabeculated and the mucosal surface is free from exudate.

The testes are small and their cut surfaces darker brown than normal. The prostate is small. The seminal vesicles are normal. The gastro-intestinal tract is normal save for melanosis of the cecum and ascending colon.

### MICROSCOPIC FINDINGS

*Bone Marrow from Sternum, Ribs and Vertebrae:* Masses of cells having the morphology of plasma cells are present and among them are many with two or more nuclei. About the capillaries these cells form a single palisade-like layer. About the tumor cells is a stroma of moderately dense connective tissue in which are many delicate collagen fibrils. Large blood vessels are not numerous but in the walls of some amyloid is demonstrated with methyl violet.

*Rib:* The muscle fibers and adipose tissue attached to the external surface show extensive amyloid changes similar to those to be described below.

*Masses about Shoulders and in the Striated Muscles:* In places many normal muscle fibers remain, but there are also many which

At the right sternoclavicular joint the bones are firmly bound together by dense white tissue. On section the sternal portion of the right clavicle and the attached segment of the manubrium contain in their marrow cavity firm grayish masses, that in the clavicle measuring about 3 cm. in length, while the remainder of the cavity contains only slightly reddish fat. The lower ends of the cervical muscles attached in the region of the right sternoclavicular joint are pale.

The mass in the left flank lies in the oblique muscles of the antero-lateral abdominal wall, extending medially to the lateral border of the left rectus muscle, and it fills the space between the iliac crest and the lower costal margin. The skin over this mass is not unusually adherent to it and there is no extension of the tumor-like growth through the underlying parietal peritoneum. The mass is easily removed from the crest of the ilium, leaving no perceptible irregularity of the innominate bone in the region of attachment. After removal the mass measures roughly 15 by 10 by 6 cm. On its external surface is a thin layer of striated muscle, below which is a zone 2.5 to 3 cm. thick similar in appearance to the lardaceous substance about the shoulder joints. Internal to this is a layer of pale muscle fibers stippled with hemorrhages, but many fibers appear to be replaced or widely separated by the firm white substance. Internal to this band is still another layer of white homogeneous material 1 cm. thick, with a few pale muscle fibers scattered through it.

A section from the swelling on the left thigh is similar in appearance to the masses about the shoulders. The mass on the antero-medial aspect of the right thigh lies in the subcutaneous fat. Its cut surface is pearly white and firm.

Sections from the tumor-like masses, treated with iodine, turn a mahogany brown color.

The ribs are free from nodular enlargements or fractures. Section through one discloses a pale brownish red fatty marrow. The bodies of the second, third and fourth lumbar vertebrae are mottled by irregular, whitish, soft masses varying in size up to 1 cm.

The heart is essentially normal. The aorta presents a mild degree of atheroma. The lungs show partial atelectasis of their lower lobes. Thyroid normal.

The spleen weighs 220 gm. The capsule is slightly thickened, the pulp reddish brown and not unusual.

the portal spaces and in those arteries adjacent to the capsule. Smaller deposits of it appear in the walls of some of the veins. None is seen about the sinusoids.

*Pancreas:* Amyloid occurs both in the arteries and veins of the interlobular septa and in the arterioles of some of the lobules. At the periphery of a few of the pancreatic lobules delicate, anastomosing fibrils of the substance surround a few of the acini. In the peripancreatic adipose tissue the fat cells are surrounded by thin mantles of amyloid and the larger blood vessels contain a subendothelial deposit.

*Adrenal:* Practically the entire fascicular zone is replaced by amyloid and it is also seen in the vessel walls in the surrounding fat.

*Kidney:* Amyloid is abundant in the capillary tufts of many of the glomeruli and is widely distributed throughout the arterioles and capillaries in other parts of the kidney. Less commonly it appears in the basement membrane of the tubules. Practically all the glomeruli have undergone sclerotic changes, many of them being reduced to small hyaline scars. The tubules are either atrophic or are dilated and filled with casts, about some of which lie giant cells. Calcium is also present in some of the tubular lumina. The interstitial tissue of the cortex is condensed in many places and is infiltrated with lymphocytes and plasma cells. About the capillaries and medium-sized arteries in the peripelvic fat amyloid is abundant.

*Testis:* Spermatogenesis is not in evidence, many of the cells in the tubules being those of the Sertoli type. In the blood vessels in the interstitial tissue and in the membrana propria of many of the tubules amyloid is present. It occurs also in the walls of the blood vessels supplying the tunica albuginea.

*Seminal Vesicles:* No amyloid found.

*Prostate:* Amyloid has infiltrated not only the walls of the blood vessels, but it appears beneath the epithelium of the prostatic glands, in places filling the interglandular stroma with coarse parallel or anastomosing fibrils. It occurs also in groups of fibers in many of the smooth muscle bundles in the gland, and it surrounds the capillaries traversing several periprostatic sympathetic ganglia.

*Stomach and Duodenum:* The section includes the pyloric end of the stomach and the adjoining part of the duodenum. Methyl violet demonstrates amyloid about the capillaries and in the stroma of the gastric and duodenal mucosa where it partially or completely

are necrotic, vacuolated or atrophic. Others show waxy degeneration. The sarcolemma nuclei of occasional necrotic individual fibers have proliferated, forming large nuclear masses at the ends of the fibers. Between the muscle cells are trabeculae of amyloid, giving characteristic staining reactions with Congo red and methyl violet. Between longitudinally cut fibers these amyloid strands run parallel to the long axes of the fibers, while in those cut transversely amyloid forms a delicate anastomosing network between adjacent fibers, often appearing between them in two delicate bands, as if deposited beneath the endothelium of the capillaries. No amyloid is found in the muscle cells themselves. In regions where the amyloid deposition is more abundant the muscle fibers are widely separated by small and heavy strands and irregular masses of amyloid. In many regions no muscle fibers are present, but the entire mass is composed of amyloid arranged in masses or clumps or in delicate or coarse anastomosing bands, the whole presenting a reticular or "porous" structure. Moderate numbers of capillaries and thin-walled blood vessels are present, and elongate nuclei suggesting morphologically those of connective tissue cells are scattered through the amyloid-containing areas. A few multinucleated giant cells, some of them partially surrounding amyloid particles, occur. In some of the sections are islands of adipose tissue.

*Mass from the Right Glenoid Fossa:* The mass is composed mainly of amyloid, together with a small amount of dense connective tissue in which are many blood vessels.

*Heart:* Amyloid has been deposited in the walls of both arteries and veins, forming in places heavy masses which markedly narrow the lumina. It also surrounds many of the cardiac fibers, appearing about individual fibers as a thin mantle. In some instances it has been deposited in the fibers themselves and it appears in small masses beneath the endocardium. In one area there has been widespread necrosis of the cardiac fibers with an infiltration of polymorphonuclear leucocytes about them.

*Aorta:* In the middle third of the media is a fine deposit of calcium.

*Spleen:* Amyloid occurs in the walls of the central arterioles and about some of the capillaries. In the malpighian bodies it forms irregular anastomosing masses and strands.

*Liver:* Amyloid deposition is heavy in the walls of the arteries of